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(54) Title: COMPOUNDS AND METHODS FOR THERAPY AND DIAGNOSIS OF LUNG CANCER

(57) Abstract

Compounds and methods for the treatment and diagnosis of lung cancer are provided. The inventive compounds include polypeptides containing at least a portion of a lung tumor protein. Vaccines and pharmaceutical compositions for immunotherapy of lung cancer comprising such polypeptides, or DNA molecules encoding such polypeptides, are also provided, together with DNA molecules for preparing the inventive polypeptides.

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COMPOUNDS AND METHODS FOR THERAPY AND DIAGNOSIS OF LUNG CANCER

TECHNICAL FIELD

The present invention relates generally to compositions and methods for the treatment and diagnosis of lung cancer. The invention is more specifically related to nucleotide sequences that are preferentially expressed in lung tumor tissue, together with polypeptides encoded by such nucleotide sequences. The inventive nucleotide sequences and polypeptides may be used in vaccines and pharmaceutical compositions for the treatment and diagnosis of lung cancer.

BACKGROUND OF THE INVENTION

Lung cancer is the primary cause of cancer death among both men and women in the U.S., with an estimated 172,000 new cases being reported in 1994. The five-year survival rate among all lung cancer patients, regardless of the stage of disease at diagnosis, is only 13%. This contrasts with a five-year survival rate of 46% among cases detected while the disease is still localized. However, only 16% of lung cancers are discovered before the disease has spread.

Early detection is difficult since clinical symptoms are often not seen until the disease has reached an advanced stage. Currently, diagnosis is aided by the use of chest x-rays, analysis of the type of cells contained in sputum and fiberoptic examination of the bronchial passages. Treatment regimens are determined by the type and stage of the cancer, and include surgery, radiation therapy and/or chemotherapy. In spite of considerable research into therapies for the disease, lung cancer remains difficult to treat.

Accordingly, there remains a need in the art for improved vaccines, treatment methods and diagnostic techniques for lung cancer.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compounds and methods for the therapy of lung cancer. In a first aspect, isolated polynucleotide molecules encoding lung

tumor polypeptides are provided, such polynucleotide molecules comprising a nucleotide sequence selected from the group consisting of: (a) sequences provided in SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30, 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157, 158, 160, 167, 168 and 171; (b) sequences complementary to a sequence provided in SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30, 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157, 158, 160, 167, 168 and 171; and (b) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

In a second aspect, isolated polypeptides are provided that comprise at least an immunogenic portion of a lung tumor protein or a variant thereof. In specific embodiments, such polypeptides comprise an amino acid sequence encoded by a polynucleotide molecule comprising a nucleotide sequence selected from the group consisting of (a) sequences recited in SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30, 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157, 158, 160, 167, 168 and 171; (b) sequences complementary to a sequence provided in SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30, 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157, 158, 160, 167, 168 and 171; and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

In related aspects, expression vectors comprising the inventive polynucleotide molecules, together with host cells transformed or transfected with such expression vectors are provided. In preferred embodiments, the host cells are selected from the group consisting of *E. coli*, yeast and mammalian cells.

In another aspect, fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known lung tumor antigen, are provided.

The present invention further provides pharmaceutical compositions comprising one or more of the above polypeptides, fusion proteins or polynucleotide molecules and a physiologically acceptable carrier, together with vaccines comprising one or

more such polypeptides, fusion proteins or polynucleotide molecules in combination with an immune response enhancer.

In related aspects, the present invention provides methods for inhibiting the development of lung cancer in a patient, comprising administering to a patient an effective amount of at least one of the above pharmaceutical compositions and/or vaccines.

Additionally, the present invention provides methods for immunodiagnosis of lung cancer, together with kits for use in such methods. Polypeptides are disclosed which comprise at least an immunogenic portion of a lung tumor protein or a variant of said protein that differs only in conservative substitutions and/or modifications, wherein the lung tumor protein comprises an amino acid sequence encoded by a polynucleotide molecule having a sequence selected from the group consisting of nucleotide sequences recited in SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171, and variants thereof. Such polypeptides may be usefully employed in the diagnosis and monitoring of lung cancer.

In one specific aspect of the present invention, methods are provided for detecting lung cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to one of the above polypeptides; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent. In preferred embodiments, the binding agent is an antibody, most preferably a monoclonal antibody.

In related aspects, methods are provided for monitoring the progression of lung cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to one of the above polypeptides; (b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent; (c) repeating steps (a) and (b); and comparing the amounts of polypeptide detected in steps (b) and (c).

Within related aspects, the present invention provides antibodies, preferably monoclonal antibodies, that bind to the inventive polypeptides, as well as diagnostic kits comprising such antibodies, and methods of using such antibodies to inhibit the development of lung cancer.

The present invention further provides methods for detecting lung cancer comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with a first and a second oligonucleotide primer in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide molecule that encodes one of the above polypeptides; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the first and second oligonucleotide primers. In a preferred embodiment, at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a polynucleotide molecule including a sequence selected from the group consisting of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171.

In a further aspect, the present invention provides a method for detecting lung cancer in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide molecule that encodes one of the above polypeptides; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. Preferably, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide molecule having a partial sequence selected from the group consisting of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154,157, 158, 160, 162-164, 167, 168 and 171.

In related aspects, diagnostic kits comprising the above oligonucleotide probes or primers are provided.

In yet a further aspect, methods for the treatment of lung cancer in a patient are provided, the methods comprising obtaining PBMC from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of lung cancer that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting cells to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells and macrophages. Compositions for the treatment of lung cancer comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the

present invention are also provided. These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the therapy and diagnosis of lung cancer. The compositions described herein include polypeptides, fusion proteins and polynucleotide molecules. Also included within the present invention are molecules (such as an antibody or fragment thereof) that bind to the inventive polypeptides. Such molecules are referred to herein as "binding agents."

In one aspect, the subject invention discloses polypeptides comprising an immunogenic portion of a human lung tumor protein, wherein the lung tumor protein includes an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO: 1-109, , 111, 113 115-151, 153, 154,157, 158, 160, 162-164, 167, 168 and 171, (b) the complements of said nucleotide sequences, and (c) variants of such sequences. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins, wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising a portion of one of the above lung tumor proteins may consist entirely of the portion, or the portion may be present within a larger polypeptide that contains additional sequences. The additional sequences may be derived from the native protein or may be heterologous, and such sequences may (but need not) be immunoreactive and/or antigenic. As detailed below, such polypeptides may be isolated from lung tumor tissue or prepared by synthetic or recombinant means.

As used herein, an "immunogenic portion" of a lung tumor protein is a portion that is capable of eliciting an immune response in a patient inflicted with lung cancer and as such binds to antibodies present within sera from a lung cancer patient. Such immunogenic portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and most preferably at least about 20 amino acid residues. Immunogenic portions of the proteins described herein may be identified in antibody binding assays. Such assays

may generally be performed using any of a variety of means known to those of ordinary skill in the art, as described, for example, in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988. For example, a polypeptide may be immobilized on a solid support (as described below) and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I-labeled Protein A. Alternatively, a polypeptide may be used to generate monoclonal and polyclonal antibodies for use in detection of the polypeptide in blood or other fluids of lung cancer patients. Methods for preparing and identifying immunogenic portions of antigens of known sequence are well known in the art and include those summarized in Paul, *Fundamental Immunology*, 3rd ed., Raven Press, 1993, pp. 243-247.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotides. A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the therapeutic, antigenic and/or immunogenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. Polypeptide variants preferably

exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as describe below) to the identified polypeptides.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also, or alternatively, contain other modifications. including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example. a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A nucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (*DNA, 2*:183, 1983). Nucleotide variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity (determined as described below) to the recited sequence.

The antigens provided by the present invention include variants that are encoded by polynucleotide sequences which are substantially homologous to one or more of the polynucleotide sequences specifically recited herein. "Substantial homology," as used herein, refers to polynucleotide sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a

solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing polynucleotide sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode an immunogenic polypeptide that is encoded by a hybridizing polynucleotide sequence.

Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer *CABIOS 5*:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space *CABIOS 4*:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees *Mol. Biol. Evol. 4*:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks *Proc. Natl. Acad., Sci. USA 80*:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent. or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Also included in the scope of the present invention are alleles of the genes encoding the nucleotide sequences recited in herein. As used herein, an "allele" or "allellic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence.

For lung tumor polypeptides with immunoreactive properties, variants may, alternatively, be identified by modifying the amino acid sequence of one of the above polypeptides, and evaluating the immunoreactivity of the modified polypeptide. For lung tumor polypeptides useful for the generation of diagnostic binding agents, a variant may be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of lung cancer. Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

The lung tumor polypeptides of the present invention, and polynucleotide molecules encoding such polypeptides, may be isolated from lung tumor tissue using any of a variety of methods well known in the art. Polynucleotide sequences corresponding to a gene

(or a portion thereof) encoding one of the inventive lung tumor proteins may be isolated from a lung tumor cDNA library using a subtraction technique as described in detail below. Examples of such polynucleotide sequences are provided in SEQ ID NO: 1-109,111,113 115-151, 153. 154, 157, 158, 160, 162-164, 167, 168 and 171. Partial polynucleotide sequences thus obtained may be used to design oligonucleotide primers for the amplification of full-length polynucleotide sequences from a human genomic DNA library or from a lung tumor cDNA library in a polymerase chain reaction (PCR), using techniques well known in the art (see, for example, Mullis et al., *Cold Spring Harbor Symp. Quant. Biol. 51*:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989). For this approach, sequence-specific primers may be designed based on the nucleotide sequences provided herein and may be purchased or synthesized.

An amplified portion may be used to isolate a full length gene from a suitable library (*e.g.*, a lung tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length

cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using techniques well known in the art (*see*, for example, Mullis et al., *Cold Spring Harbor Symp. Quant. Biol. 51*:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989). and software well known in the art may also be employed. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia et al., *Nucl. Acids Res. 16*:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Additional techniques include capture PCR (Lagerstrom et al., *PCR Methods Applic. 1*:111-19, 1991) and walking PCR (Parker et al., *Nucl. Acids. Res. 19*:3055-60, 1991). Transcription-Mediated Amplification, or TMA is another method that may be utilized for the amplification of DNA, rRNA, or mRNA, as described in Patent No. PCT/US91/03184. This autocatalytic and isothermic non-PCR based method utilizes two primers and two enzymes: RNA polymerase and reverse transcriptase. One primer contains a promoter sequence for RNA polymerase. In the first amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3'end of the promoter-primer. The

RNA in the resulting complex is degraded and a second primer binds to the DNA copy. A new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating double stranded DNA. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to the expotential expansion of the RNA amplicon. Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence.

Once a polynucleotide sequence encoding a polypeptide is obtained, the polypeptide may be produced recombinantly by inserting the polynucleotide sequence into an expression vector and expressing the polypeptide in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polynucleotide molecule that encodes the recombinant polypeptide. Suitable host cells include prokaryotes, yeast, insect and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as COS or CHO cells. The polynucleotide sequences expressed in this manner may encode naturally occurring polypeptides, portions of naturally occurring polypeptides, or other variants thereof. Supernatants from suitable host/vector systems which secrete the recombinant polypeptide may first be concentrated using a commercially available filter. The concentrate may then be applied to a suitable purification matrix, such as an affinity matrix or ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify the recombinant polypeptide.

The lung tumor polypeptides disclosed herein may also be generated by synthetic means. In particular, synthetic polypeptides having fewer than about 100 amino

acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain (see, for example, Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA). and may be operated according to the manufacturer's instructions.

In addition, lung tumor antigens may be identified by T cell expression cloning. One source of tumor specific T cells is from surgically excised tumors from human patients. In one method for isolating and characterizing tumor specific T cells. the excised tumor is minced and enzymatically digested for several hours to release tumor cells and infiltrating lymphocytes (tumor infiltrating T cells, or TILs). The cells are washed in HBSS buffer and passed over a Ficoll (100%/75%/HBSS) discontinuous gradient to separate tumor cells and lymphocytes from non-viable cells. Two bands are harvested from the interfaces; the upper band at the 75%/HBSS interface contains predominantly tumor cells. while the lower band at the 100%/75%/HBSS interface contains a majority of lymphocytes. The TILs are expanded in culture by techniques well known in the art, but preferably in culture media supplemented with 10 ng/ml IL-7 and 100 U/ml IL-2, or alternatively, cultured and expanded in tissue culture plates that have been pre-adsorbed with anti-CD3 monoclonal antibody (OKT3). The resulting TIL cultures are analyzed by FACS to confirm that the vast majority are CD8+ T cells (>90% of gated population).

In addition, the tumor cells are also expanded in culture using standard techniques well known in the art to establish a tumor cell line, which is later confirmed to be lung carcinoma cells by immunohistochemical analysis. The tumor cell line is transduced with a retroviral vector to express human CD80. The tumor cell line is further characterized by FACS analysis to confirm the strong expression levels of CD80, class I and II MHC molecules.

The specificity of the TIL lines to lung tumor is confirmed by INF-γ and/or TNF-α cytokine release assays. For example, TIL cells from day 21 cultures are co-cultured

with either autologous or allogeneic tumor cells, EBV-immortalized LCL, or control cell lines Daudi and K562 and the culture supernatant monitored by ELISA for the presence of cytokines. The expression of these specific cytokines in the presence of tumor or negative control cells indicates whether the TIL lines are tumor specific and potentially recognizing tumor antigen presented by the autologous MHC molecules.

The characterized tumor-specific TIL lines can be expanded and cloned by methods well known in the art. For example, the TIL lines may be expanded to suitable numbers for T cell expression cloning by using soluble anti-CD3 antibody in culture with irradiated EBV transformed LCLs and PBL feeder cells in the presence of 20 U/ml IL-2. Clones from the expanded TIL lines can be generated by standard limiting dilution techniques. In particular, TIL cells are seeded at 0.5 cells/well in a 96-well U bottom plate and stimulated with CD-80-transduced autologous tumor cells, EBV transformed LCL, and PBL feeder cells in the presence of 50 U/ml IL-2. These clones may be further analyzed for tumor specificity by ^{51}Cr microcytotoxicity and IFN-γ bioassays. Additionally, the MHC restriction element recognized by the TIL clones may be determined by antibody blocking studies well known in the art.

The CTL lines or clones described above may be employed to identify tumor specific antigens. For example, autologous fibroblasts or LCL from a patient may be transfected or transduced with polynucleotide fragments derived from a lung tumor cDNA library to generate target cells expressing tumor polypeptides. The target cells expressing tumor polypeptides in the context of MHC will be recognized by the CTL line or clone resulting in T-cell activation, which can be monitored by cytokine detection assays. The tumor gene being expressed by the target cell and recognized by the tumor-specific CTL is then isolated by techniques described above. In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure form (*i.e.*, the polypeptides are homogenous as determined by amino acid composition and primary sequence analysis). Preferably, the polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. In certain preferred embodiments, described in more detail below, the substantially pure polypeptides

are incorporated into pharmaceutical compositions or vaccines for use in one or more of the methods disclosed herein.

In a related aspect, the present invention provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known lung tumor antigen, together with variants of such fusion proteins. The fusion proteins of the present invention may (but need not) include a linker peptide between the first and second polypeptides.

A polynucleotide sequence encoding a fusion protein of the present invention is constructed using known recombinant DNA techniques to assemble separate polynucleotide sequences encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. Peptide sequences are not required when the first and second

polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated polynucleotide sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of polynucleotide are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons require to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided that comprise a polypeptide of the present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, for example, Stoute et al. *New Engl. J. Med.*, *336*:86-91 (1997)).

Polypeptides of the present invention that comprise an immunogenic portion of a lung tumor protein may generally be used for therapy of lung cancer, wherein the polypeptide stimulates the patient's own immune response to lung tumor cells. The present invention thus provides methods for using one or more of the compounds described herein (which may be polypeptides, polynucleotide molecules or fusion proteins) for immunotherapy of lung cancer in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with disease, or may be free of detectable disease. Accordingly, the compounds disclosed herein may be used to treat lung cancer or to inhibit the development of lung cancer. The compounds are preferably administered either prior to or following surgical removal of primary tumors and/or treatment by administration of radiotherapy and conventional chemotherapeutic drugs.

In these aspects, the inventive polypeptide is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. The vaccines may comprise one or more such polypeptides and a non-specific immune-response enhancer, wherein the non-specific immune response enhancer is capable of eliciting or enhancing an immune response to an exogenous antigen. Examples of non-specific-immune response enhancers include

adjuvants, biodegradable microspheres (*e.g.*, polylactic galactide) and liposomes (into which the polypeptide is incorporated). Pharmaceutical compositions and vaccines may also contain other epitopes of lung tumor antigens, either incorporated into a fusion protein as described above (*i.e.*, a single polypeptide that contains multiple epitopes) or present within a separate polypeptide.

Alternatively, a pharmaceutical composition or vaccine may contain polynucleotide encoding one or more of the above polypeptides and/or fusion proteins, such that the polypeptide is generated *in situ*. In such pharmaceutical compositions and vaccines, the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an epitope of a lung cell antigen on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., *PNAS 86*:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci. 569*:86-103, 1989; Flexner et al., *Vaccine 8*:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques 6*:616-627, 1988; Rosenfeld et al., *Science 252*:431-434, 1991; Kolls et al., *PNAS 91*:215-219, 1994; Kass-Eisler et al., *PNAS 90*:11498-11502, 1993; Guzman et al., *Circulation 88*:2838-2848, 1993; and Guzman et al., *Cir. Res. 73*:1202-1207, 1993. Techniques for incorporating polynucleotide into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be "naked," as described, for example, in published PCT application WO 90/11092, and Ulmer et al., *Science 259*:1745-1749, 1993, reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked polynucleotides may be increased by coating the polynucleotides onto biodegradable beads, which are efficiently transported into the cells.

Routes and frequency of administration, as well as dosage, will vary from individual to individual and may parallel those currently being used in immunotherapy of other diseases. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 10 doses may be administered over a 3-24 week period. Preferably, 4 doses are administered, at an interval of 3 months, and booster administrations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or polynucleotide that is effective to raise an immune response (cellular and/or humoral) against lung tumor cells in a treated patient. A suitable immune response is at least 10-50% above the basal (*i.e.*, untreated) level. In general, the amount of polypeptide present in a dose (or produced *in situ* by the polynucleotide molecule(s) in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.01 mL to about 5 mL.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a lipid, a wax and/or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and/or magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic glycolide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of immune-response enhancers may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune response, such as lipid A, *Bordella pertussis* or *Mycobacterium tuberculosis*. Such adjuvants are commercially

available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ).Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of cancer. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (for example, tumor vaccines, bacterial adjuvants, and/or cytokines).

In passive immunotherapy, treatment involves the delivery of biologic reagents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper, gamma/delta T lymphocytes, tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4,918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells *in vitro*. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. These *in vitro* culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a variety of standard techniques well known in the art. For example, antigen presenting cells may be transfected or transduced with a polynucleotide sequence,

wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipid-mediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term *in vivo*. Studies have demonstrated that cultured T-cells can be induced to grow *in vivo* and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., *et al*, "Therapy With Cultured T Cells: Principles Revisited," *Immunological Reviews, 157*:177, 1997).

The polypeptides disclosed herein may also be employed to generate and/or isolate tumor-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ CTL clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate tumor reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigen-specific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al, (Crit. Rev. Oncol. Hematol., 22*(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as CellPro Incorporated's (Bothell, WA) CEPRATE™ system (see U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of tumor antigen-specific T cells is then expanded using

standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the polypeptides disclosed herein can be cloned, expanded, and transferred into other vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may be transfected with the appropriate genes to express the variable domains from tumor specific monoclonal antibodies as the extracellular recognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-independent manner. See for example. Eshhar, Z., *Cancer Immunol Immunother*, 45(3-4):131-6, 1997 and Hwu, P., et al, *Cancer Res*, 55(15):3369-73, 1995. Another embodiment may include the transfection of tumor antigen specific alpha and beta T cell receptor chains into alternate T cells, as in Cole. DJ, et al, *Cancer Res*, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate tumors in a murine model has been demonstrated by Cheever et al, *Immunological Reviews, 157*:177, 1997).

Furthermore, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient.

Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient. Polypeptides and fusion proteins of the present invention may also, or alternatively, be used to generate binding agents, such as antibodies or fragments thereof, that are capable of detecting metastatic human lung tumors. Binding agents of the present invention may generally be prepared using methods known to those of ordinary skill in the art, including the representative procedures described herein.

Binding agents are capable of differentiating between patients with and without lung cancer, using the representative assays described herein. In other words, antibodies or other binding agents raised against a lung tumor protein, or a suitable portion thereof, will generate a signal indicating the presence of primary or metastatic lung cancer in at least about 20% of patients afflicted with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without primary or metastatic lung cancer. Suitable portions of such lung tumor proteins are portions that are able to generate a binding agent that indicates the presence of primary or metastatic lung cancer in substantially all (*i.e.*, at least about 80%, and preferably at least about 90%) of the patients for which lung cancer would be indicated using the full length protein, and that indicate the absence of lung cancer in substantially all of those samples that would be negative when tested with full length protein. The representative assays described below, such as the two-antibody sandwich assay, may generally be employed for evaluating the ability of a binding agent to detect metastatic human lung tumors.

The ability of a polypeptide prepared as described herein to generate antibodies capable of detecting primary or metastatic human lung tumors may generally be evaluated by raising one or more antibodies against the polypeptide (using, for example, a representative method described herein) and determining the ability of such antibodies to detect such tumors in patients. This determination may be made by assaying biological samples from patients with and without primary or metastatic lung cancer for the presence of a polypeptide that binds to the generated antibodies. Such test assays may be performed, for example, using a representative procedure described below. Polypeptides that generate antibodies capable of detecting at least 20% of primary or metastatic lung tumors by such procedures are considered to be useful in assays for detecting primary or metastatic human lung tumors. Polypeptide specific antibodies may be used alone or in combination to improve sensitivity.

Polypeptides capable of detecting primary or metastatic human lung tumors may be used as markers for diagnosing lung cancer or for monitoring disease progression in patients. In one embodiment, lung cancer in a patient may be diagnosed by evaluating a biological sample obtained from the patient for the level of one or more of the above

polypeptides, relative to a predetermined cut-off value. As used herein, suitable "biological samples" include blood, sera, urine and/or lung secretions.

The level of one or more of the above polypeptides may be evaluated using any binding agent specific for the polypeptide(s). A "binding agent," in the context of this invention, is any agent (such as a compound or a cell) that binds to a polypeptide as described above. As used herein, "binding" refers to a noncovalent association between two separate molecules (each of which may be free (*i.e.*, in solution) or present on the surface of a cell or a solid support), such that a "complex" is formed. Such a complex may be free or immobilized (either covalently or noncovalently) on a support material. The ability to bind may generally be evaluated by determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind" in the context of the present invention when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known to those of ordinary skill in the art.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome with or without a peptide component, an RNA molecule or a peptide. In a preferred embodiment, the binding partner is an antibody, or a fragment thereof. Such antibodies may be polyclonal, or monoclonal. In addition, the antibodies may be single chain, chimeric, CDR-grafted or humanized. Antibodies may be prepared by the methods described herein and by other methods well known to those of skill in the art.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding partner to detect polypeptide markers in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. In a preferred embodiment, the assay involves the use of binding partner immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a second binding partner that contains a reporter group. Suitable second binding partners include antibodies that bind to the binding partner/polypeptide complex. Alternatively, a competitive assay may be utilized, in which a

polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding partner after incubation of the binding partner with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding partner is indicative of the reactivity of the sample with the immobilized binding partner.

The solid support may be any material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 μg, and preferably about 100 ng to about 1 μg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a second antibody (containing a reporter group) capable of binding to a different site on the polypeptide is added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with lung cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of antibody to reporter group may be achieved using standard methods known to those of ordinary skill in the art.

The second antibody is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound second antibody is then removed and bound second antibody is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of lung cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without lung cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for lung cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for lung cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the antibody is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized antibody as the sample passes through the membrane. A second, labeled antibody then binds to the antibody-polypeptide complex as a solution containing the second antibody flows through the membrane. The detection of bound second antibody may then be performed as described above. In the strip test format, one end of the membrane to which antibody is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second antibody and to the area of immobilized antibody. Concentration of second antibody at the area of immobilized antibody indicates the presence of lung cancer. Typically, the concentration of second antibody at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of antibody immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the antigens or antibodies of the present invention. The above descriptions are intended to be exemplary only.

In another embodiment, the above polypeptides may be used as markers for the progression of lung cancer. In this embodiment, assays as described above for the diagnosis of lung cancer may be performed over time, and the change in the level of reactive polypeptide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, lung cancer is progressing in those patients in whom the level of polypeptide detected by the binding agent increases over time. In contrast, lung cancer is not progressing when the level of reactive polypeptide either remains constant or decreases with time.

Antibodies for use in the above methods may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* *6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield,

such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Monoclonal antibodies of the present invention may also be used as therapeutic reagents, to diminish or eliminate lung tumors. The antibodies may be used on their own (for instance, to inhibit metastases) or coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y, ^{123}I, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the

catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing

nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

Diagnostic reagents of the present invention may also comprise polynucleotide sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify lung tumor-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a polynucleotide molecule encoding a lung tumor protein of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a polynucleotide molecule encoding a lung tumor protein of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

As used herein, the term "oligonucleotide primer/probe specific for a polynucleotide molecule" means an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to the polynucleotide molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a polynucleotide molecule comprising sequence selected from SEQ ID NO: 1-109, 111, 113 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a polynucleotide molecule comprising a sequence provided in SEQ ID NO: 1-109,111, 113 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171. Techniques for both PCR based assays and hybridization assays are

well known in the art (see, for example, Mullis *et al. Ibid*; Ehrlich, *Ibid*). Primers or probes may thus be used to detect lung tumor-specific sequences in biological samples, including blood, semen, lung tissue and/or lung tumor tissue.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

ISOLATION AND CHARACTERIZATION OF cDNA SEQUENCES ENCODING LUNG TUMOR POLYPEPTIDES

This example illustrates the isolation of cDNA molecules encoding lung tumor-specific polypeptides from lung tumor cDNA libraries.

A. Isolation of cDNA Sequences from a Lung Squamous Cell Carcinoma Library

A human lung squamous cell carcinoma cDNA expression library was constructed from poly A^+ RNA from a pool of two patient tissues using a Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL Life Technologies, Gaithersburg, MD) following the manufacturer's protocol. Specifically, lung carcinoma tissues were homogenized with polytron (Kinematica, Switzerland) and total RNA was extracted using Trizol reagent (BRL Life Technologies) as directed by the manufacturer. The poly A^+ RNA was then purified using an oligo dT cellulose column as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. First-strand cDNA was synthesized using the NotI/Oligo-dT18 primer. Double-stranded cDNA was synthesized, ligated with BstXI/EcoRI adaptors (Invitrogen, San Diego, CA) and digested with NotI. Following size fractionation with cDNA size fractionation columns (BRL Life Technologies), the cDNA was ligated into the BstXI/NotI

site of pcDNA3.1 (Invitrogen) and transformed into ElectroMax *E. coli* DH10B cells (BRL Life Technologies) by electroporation.

Using the same procedure, a normal human lung cDNA expression library was prepared from a pool of four tissue specimens. The cDNA libraries were characterized by determining the number of independent colonies, the percentage of clones that carried insert, the average insert size and by sequence analysis. The lung squamous cell carcinoma library contained 2.7×10^6 independent colonies, with 100% of clones having an insert and the average insert size being 2100 base pairs. The normal lung cDNA library contained 1.4×10^6 independent colonies, with 90% of clones having inserts and the average insert size being 1800 base pairs. For both libraries, sequence analysis showed that the majority of clones had a full length cDNA sequence and were synthesized from mRNA

cDNA library subtraction was performed using the above lung squamous cell carcinoma and normal lung cDNA libraries, as described by Hara *et al.* (*Blood*, *84*:189-199, 1994) with some modifications. Specifically, a lung squamous cell carcinoma-specific subtracted cDNA library was generated as follows. Normal tissue cDNA library (80 μg) was digested with BamHI and XhoI, followed by a filling-in reaction with DNA polymerase Klenow fragment. After phenol-chloroform extraction and ethanol precipitation, the DNA was dissolved in 133 μl of H_2O, heat-denatured and mixed with 133 μl (133 μg) of Photoprobe biotin (Vector Laboratories, Burlingame, CA). As recommended by the manufacturer, the resulting mixture was irradiated with a 270 W sunlamp on ice for 20 minutes. Additional Photoprobe biotin (67 μl) was added and the biotinylation reaction was repeated. After extraction with butanol five times, the DNA was ethanol-precipitated and dissolved in 23 μl H_2O to form the driver DNA.

To form the tracer DNA, 10 μg lung squamous cell carcinoma cDNA library was digested with NotI and SpeI, phenol chloroform extracted and passed through Chroma spin-400 columns (Clontech, Palo Alto, CA). Typically, 5 μg of cDNA was recovered after the sizing column. Following ethanol precipitation, the tracer DNA was dissolved in 5 μl H_2O. Tracer DNA was mixed with 15 μl driver DNA and 20 μl of 2 x hybridization buffer (1.5 M NaCl/10 mM EDTA/50 mM HEPES pH 7.5/0.2% sodium dodecyl sulfate), overlaid with mineral oil, and heat-denatured completely. The sample was immediately transferred

into a 68 °C water bath and incubated for 20 hours (long hybridization [LH]). The reaction mixture was then subjected to a streptavidin treatment followed by phenol/chloroform extraction. This process was repeated three more times. Subtracted DNA was precipitated, dissolved in 12 µl H_2O, mixed with 8 µl driver DNA and 20 µl of 2 x hybridization buffer, and subjected to a hybridization at 68 °C for 2 hours (short hybridization [SH]). After removal of biotinylated double-stranded DNA, subtracted cDNA was ligated into NotI/SpeI site of chloramphenicol resistant pBCSK$^+$ (Stratagene, La Jolla, CA) and transformed into ElectroMax *E. coli* DH10B cells by electroporation to generate a lung squamous cell carcinoma specific subtracted cDNA library (herein after referred to as "lung subtraction I").

A second lung squamous cell carcinoma specific subtracted cDNA library (referred to as "lung subtraction II") was generated in a similar way to the lung subtraction library I, except that eight frequently recovered genes from lung subtraction I were included in the driver DNA, and 24,000 independent clones were recovered.

To analyze the subtracted cDNA libraries, plasmid DNA was prepared from 320 independent clones, randomly picked from the subtracted lung squamous cell carcinoma specific libraries. Representative cDNA clones were further characterized by DNA sequencing with a Perkin Elmer/Applied Biosystems Division Automated Sequencer Model 373A and/or Model 377 (Foster City, CA). The cDNA sequences for sixty isolated clones are provided in SEQ ID NO: 1-60. These sequences were compared to known sequences in the gene bank using the EMBL and GenBank databases (release 96). No significant homologies were found to the sequences provided in SEQ ID NO: 2, 3, 19, 38 and 46. The sequences of SEQ ID NO: 1, 6-8, 10-13, 15, 17, 18, 20-27, 29, 30, 32, 34-37, 39-45, 47-49, 51, 52, 54, 55 and 57-59 were found to show some homology to previously identified expressed sequence tags (ESTs). The sequences of SEQ ID NO: 9, 28, 31 and 33 were found to show some homology to previously identified non-human gene sequences and the sequences of SEQ ID NO: 4, 5, 14, 50, 53, 56 and 60 were found to show some homology to gene sequences previously identified in humans.

The subtraction procedure described above was repeated using the above lung squamous cell carcinoma cDNA library as the tracer DNA, and the above normal lung tissue cDNA library and a cDNA library from normal liver and heart (constructed from a pool of

one sample of each tissue as described above), plus twenty other cDNA clones that were frequently recovered in lung subtractions I and II, as the driver DNA (lung subtraction III). The normal liver and heart cDNA library contained 1.76×10^6 independent colonies, with 100% of clones having inserts and the average insert size being 1600 base pairs. Ten additional clones were isolated (SEQ ID NO: 61-70). Comparison of these cDNA sequences with those in the gene bank as described above, revealed no significant homologies to the sequences provided in SEQ ID NO: 62 and 67. The sequences of SEQ ID NO: 61, 63-66, 68 and 69 were found to show some homology to previously isolated ESTs and the sequence provided in SEQ ID NO: 70 was found to show some homology to a previously identified rat gene.

B. Isolation of cDNA Sequences from a Lung Adenocarcinoma Library

A human lung adenocarcinoma cDNA expression library was constructed as described above. The library contained 3.2×10^6 independent colonies, with 100% of clones having an insert and the average insert size being 1500 base pairs. Library subtraction was performed as described above using the normal lung and normal liver and heart cDNA expression libraries described above as the driver DNA. Twenty-six hundred independent clones were recovered.

Initial cDNA sequence analysis from 100 independent clones revealed many ribosomal protein genes. The cDNA sequences for fifteen clones isolated in this subtraction are provided in SEQ ID NO: 71-86. Comparison of these sequences with those in the gene bank as described above revealed no significant homologies to the sequence provided in SEQ ID NO: 84. The sequences of SEQ ID NO: 71, 73, 74, 77, 78 and 80-82 were found to show some homology to previously isolated ESTs, and the sequences of SEQ ID NO: 72, 75, 76, 79, 83 and 85 were found to show some homology to previously identified human genes.

Example 2

DETERMINATION OF TISSUE SPECIFICITY OF LUNG TUMOR POLYPEPTIDES

Using gene specific primers, mRNA expression levels for seven representative lung tumor polypeptides described in Example 1 were examined in a variety of normal and tumor tissues using RT-PCR.

Briefly, total RNA was extracted from a variety of normal and tumor tissues using Trizol reagent as described above. First strand synthesis was carried out using 2 μg of total RNA with SuperScript II reverse transcriptase (BRL Life Technologies) at 42 °C for one hour. The cDNA was then amplified by PCR with gene-specific primers. To ensure the semi-quantitative nature of the RT-PCR, β-actin was used as an internal control for each of the tissues examined. 1 μl of 1:30 dilution of cDNA was employed to enable the linear range amplification of the β-actin template and was sensitive enough to reflect the differences in the initial copy numbers. Using these conditions, the β-actin levels were determined for each reverse transcription reaction from each tissue. DNA contamination was minimized by DNase treatment and by assuring a negative PCR result when using first strand cDNA that was prepared without adding reverse transcriptase.

mRNA Expression levels were examined in five different types of tumor tissue (lung squamous cell carcinoma from 3 patients, lung adenocarcinoma, colon tumor from 2 patients, breast tumor and prostate tumor), and thirteen different normal tissues (lung from 4 donors, prostate, brain, kidney, liver, ovary, skeletal muscle, skin, small intestine, stomach, myocardium, retina and testes). Using a 10-fold amount of cDNA, the antigen LST-S1-90 (SEQ ID NO: 3) was found to be expressed at high levels in lung squamous cell carcinoma and in breast tumor, and at low to undetectable levels in the other tissues examined.

The antigen LST-S2-68 (SEQ ID NO: 15) appears to be specific to lung and breast tumor, however, expression was also detected in normal kidney. Antigens LST-S1-169 (SEQ ID NO: 6) and LST-S1-133 (SEQ ID NO: 5) appear to be very abundant in lung tissues (both normal and tumor), with the expression of these two genes being decreased in most of the normal tissues tested. Both LST-S1-169 and LST-S1-133 were also expressed in breast and colon tumors. Antigens LST-S1-6 (SEQ ID NO: 7) and LST-S2-I2-5F (SEQ ID NO: 47) did not show tumor or tissue specific expression, with the expression of LST-S1-28 being rare and only detectable in a few tissues. The antigen LST-S3-7 (SEQ ID NO: 63) showed lung and breast tumor specific expression, with its message only being detected in

normal testes when the PCR was performed for 30 cycles. Lower level expression was detected in some normal tissues when the cycle number was increased to 35. Antigen LST-S3-13 (SEQ ID NO: 66) was found to be expressed in 3 out of 4 lung tumors. one breast tumor and both colon tumor samples. Its expression in normal tissues was lower compared to tumors, and was only detected in 1 out of 4 normal lung tissues and in normal tissues from kidney, ovary and retina. Expression of antigens LST-S3-4 (SEQ ID NO: 62) and LST-S3-14 (SEQ ID NO: 67) was rare and did not show any tissue or tumor specificity. Consistent with Northern blot analyses, the RT-PCT results on antigen LAT-S1-A-10A (SEQ ID NO: 78) suggested that its expression is high in lung, colon, stomach and small intestine tissues, including lung and colon tumors, whereas its expression was low or undetectable in other tissues.

A total of 2002 cDNA fragments isolated in lung subtractions I, II and III, described above, were colony PCR amplified and their mRNA expression levels in lung tumor, normal lung, and various other normal and tumor tissues were determined using microarray technology (Synteni, Palo Alto, CA). Briefly, the PCR amplification products were dotted onto slides in an array format, with each product occupying a unique location in the array. mRNA was extracted from the tissue sample to be tested, reverse transcribed, and fluorescent-labeled cDNA probes were generated. The microarrays were probed with the labeled cDNA probes, the slides scanned and fluorescence intensity was measured. This intensity correlates with the hybridization intensity. Seventeen non-redundant cDNA clones showed over-expression in lung squamous tumors, with expression in normal tissues tested (lung, skin, lymph node, colon, liver, pancreas, breast, heart, bone marrow, large intestine, kidney, stomach, brain, small intestine, bladder and salivary gland) being either undetectable, or 10-fold less compared to lung squamous tumors. The determined partial cDNA sequences for the clone L513S are provided in SEQ ID NO: 87 and 88; those for L514S are provided in SEQ ID NO: 89 and 90; those for L516S in SEQ ID NO: 91 and 92; that for L517S in SEQ ID NO: 93; that for L519S in SEQ ID NO: 94; those for L520S in SEQ ID NO: 95 and 96; those for L521S in SEQ ID NO: 97 and 98; that for L522S in SEQ ID NO: 99; that for L523S in SEQ ID NO: 100; that for L524S in SEQ ID NO: 101; that for L525S in SEQ ID NO: 102; that for L526S in SEQ ID NO: 103; that for L527S in SEQ ID NO: 104; that for L528S in

SEQ ID NO: 105; that for L529S in SEQ ID NO: 106; and those for L530S in SEQ ID NO: 107 and 108. Additionally, the full-length cDNA sequences for L503S and L514S (variants 1 and 2), are provided in SEQ ID NO: 151, 153 and 154, respectively, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 152, 155 and 156. Due to polymorphisms, the clone L531S appears to have two forms. A first determined full-length cDNA sequence for L531S is provided in SEQ ID NO: 109, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 110. A second determined full-length cDNA sequence for L531S is provided in SEQ ID NO: 111, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 112. The sequence of SEQ ID NO: 111 is identical to that of SEQ ID NO: 109, except that it contains a 27 bp insertion. Similarly, L514S also has two alternatively spliced forms; the first variant cDNA is listed as SEQ ID NO: 153, with the corresponding amino acid sequence as SEQ ID NO: 155. The second variant form of L514S full-length cDNA is referred to as SEQ ID NO: 154, with its corresponding amino acid sequence as SEQ ID NO: 156.

Full length cloning for L524S (SEQ ID NO: 101) yielded two variants (SEQ ID NO: 163 and 164) with the corresponding predicted amino acid sequences (SEQ ID NO: 165 and 166), respectively. Both variants have been shown to encode parathyroid hormone-related peptide.

Comparison of the sequences of L514S and L531S (SEQ ID NO: 87 and 88, 89 and 90, and 109, respectively) with those in the gene bank, as described above, revealed no significant homologies to known sequences. The sequences of L513S, L516S, L517S, L519S, L520S and L530S (SEQ ID NO: 87 and 88, 91 and 92, 93, 94, 95 and 96, 107 and 108, respectively) were found to show some homology to previously identified ESTs. The sequences of L521S, L522S, L523S, L524S, L525S, L526S, L527S, L528S and L529S (SEQ ID NO: 97 and 98, 99, 99, 101, 102, 103, 104, 105, and 106, respectively) were found to represent known genes. The determined full-length cDNA sequences for L520S is provided in SEQ ID NO: 113, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 114. Subsequent microarray analysis has shown L520S to be overexpressed in breast tumors in addition to lung squamous tumors.

Further analysis has demonstrated L529S (SEQ ID NO: 106 and 115), L525S (SEQ ID NO: 102 and 120) and L527S (SEQ ID NO: 104) are cytosleletal components and potentially squamous cell specific proteins. L529S is connexin 26, a gap junction protein. It is highly expressed in lung squamous tumor 9688T, and moderately over-expressed in two others. However, lower level expression of connexin 26 is also detectable in normal skin, colon, liver and stomach. The over-expression of connexin 26 in some breast tumors has been reported and a mutated form of L529S may result in over-expression in lung tumors. L525S is plakophilin 1, a desmosomal protein found in plaque-bearing adhering junctions of the skin. Expression levels for L525S mRNA is highly elevated in three out of four lung squamous tumors tested, and in normal skin. L527S has been identified as keratin 6 isoform, type II 58 Kd keratin, and cytokeratin 13 and shows over-expression in squamous tumors and low expression in normal skin, breast and colon tissues. Notably, keratin and keratin-related genes have been extensively documented as potential markers for lung cancer including CYFRA2.1 (Pastor, A., et al, *Eur. Respir. J.*, 10:603-609, 1997). L513S (SEQ ID NO: 87 and 88) shows moderate over-expression in several tumor tissues tested, and encodes a protein that was first isolated as a pemphigus vulgaris antigen.

L520S (SEQ ID NO: 95 and 96) and L521S (SEQ ID NO: 97 and 98) are highly expressed in lung squamous tumors, and L520S is up-regulated in normal salivary gland and L521S is over-expressed in normal skin. Both belong to a family of small proline rich proteins and represent markers for fully differentiated squamous cells. L521S has been described as a specific marker for lung squamous tumor (Hu, R., et al, *Lung Cancer*, 20:25-30, 1998). L515S (SEQ ID NO: 162) encodes IGF-β2 and L516S is an aldose reductase homologue and both are moderately expressed in lung squamous tumors and in normal colon. Notably, L516S (SEQ ID NO: 91 and 92) is up-regulated in metastatic tumors but not primary lung adenocarcinoma., an indication of its potential role in metatasis and a potential prognostic marker. L522S (SEQ ID NO: 99) is moderately over-expressed in lung squamous tumors with minimum expression in normal tissues. L522S has been shown to belong to a class IV alcohol dehydrogenase, ADH7, and its expression profile suggests it is a squamous cell specific antigen. L523S (SEQ ID NO: 100) is moderately over-expressed in lung

squamous tumor, human pancreatic cancer cell lines and pancreatic cancer tissues, suggesting this gene may be a shared antigen between pancreatic and lung squamous cell cancer.

L524S (SEQ ID NO: 101) is over-expressed in the majority of squamous tumors tested and is homolgous with parathyroid hormone-related peptide (PTHrP), which is best known to cause humoral hypercalcaemia associated with malignant tumors such as leukemia, prostate and breast cancer. It is also believed that PTHrP is most commonly associated with squamous carcinoma of lung and rarely with lung adenocarcinoma (Davidson, L.A., et al, *J. Pathol.*, 178: 398-401, 1996). L528S (SEQ ID NO: 105) is highly over-expressed in two lung squamous tumors with moderate expression in two other squamous tumors, one lung adenocarcinoma and some normal tissues, including skin, lymph nodes, heart, stomach and lung. It encodes the NMB gene that is similar to the precursor of melanocyte specific gene Pmel17, wfhich is reported to be preferentially expressed in low-metastatic potential melanoma cell lines. This suggests that L528S may be a shared antigen in both melanoma and lung aquamous cell carcinoma. L526S (SEQ ID NO: 103) is overexpressed in all lung squamous cell tumor tissues tested and has been shown to share homology with a gene (ATM) in which a mutation causes ataxia telangiectasia, a genetic disorder in humans causing a predisposition to cancer, among other symptoms. ATM encodes a protein that activates p53 mediated cell-cycle checkpoint through direct binding and phosphorylation of the p53 molecule. Approximately 40% of lung cancer is associated with p53 mutations, and it is speculated that over-expression of ATM is a result of compensation for loss of p53 function, but it is unknown whether over-expression is the cause of result of lung squamous cell carcinoma. Additionally, expression of L526S (ATM) is also detected in a metastatic but not lung adenocarcinoma, suggesting a role in metastasis.

Example 3

ISOLATION AND CHARACTERIZATION OF LUNG TUMOR POLYPEPTIDES BY PCR-BASED SUBTRACTION

Eight hundred and fifty seven clones from a cDNA subtraction library, containing cDNA from a pool of two human lung squamous tumors subtracted against eight

normal human tissue cDNAs including lung, PBMC, brain, heart, kidney, liver, pancreas, and skin, (Clontech, Palo Alto, CA) were derived and submitted to a first round of PCR amplification. This library was subjected to a second round of PCR amplification, following the manufacturer's protocol. The resulting cDNA fragments were subcloned into the vector P7- Adv vector (Clontech, Palo Alto, CA) and transformed into DH5α *E. coli* (Gibco, BRL). DNA was isolated from independent clones and sequenced using a Perkin Elmer/Applied Biosystems Division Automated Sequencer Model 373A.

One hundred and sixty two positive clones were sequenced. Comparison of the DNA sequences of these clones with those in the gene bank using the EMBL and GenBank databases, as described above, revealed no significant homologies to 13 of these clones, hereinafter referred to as Contig 13, 16, 17, 19, 22, 24, 29, 47, 49, 56-59 . The determined cDNA sequences for these clones are provided in SEQ ID NO: 125, 127-129, 131-133, 142, 144, 148-150, and 157, respectively. Contigs 1, 3-5, 7-10, 12, 11, 15, 20, 31, 33, 38, 39, 41, 43, 44, 45, 48, 50, 53, 54 (SEQ ID NO: 115-124, 126, 130, 134-141, 143, 145-147, respectively) were found to show some degree of homology to previously identified DNA sequences. Contig 57 (SEQ ID NO: 149) was found to represent the clone L519S (SEQ ID NO: 94) disclosed in US. Patent Application No. 09/123,912, filed July 27, 1998. To the best of the inventors' knowledge, none of these sequences have been previously shown to be differentially over-expressed in lung tumors.

mRNA expression levels for representative clones in lung tumor tissues, normal lung tissues (n=4), resting PBMC, salivary gland, heart, stomach, lymph nodes, skeletal muscle, soft palate, small intestine, large intestine, bronchial, bladder, tonsil, kidney, esophagus, bone marrow, colon, adrenal gland, pancreas, and skin, (all derived from human) were determined by RT-PCR as described above. Expression levels using microarray technology, as described above, were examined in one sample of each tissue type unless otherwise indicated.

Contig 3 (SEQ ID NO: 116) was found to be highly expressed in all head and neck squamous cell tumors tested (17/17), and expressed in the majority (8/12) of lung squamous tumors, (high expression in 7/12, moderate in 2/12, and low in 2/12), while showing negative expression for 2/4 normal lung tissues and low expression in the remaining

two samples. Contig 3 showed moderate expression in skin and soft palate, and lowered expression levels in resting PBMC, large intestine, salivary gland, tonsil, pancreas, esophagus, and colon. Contig 11 (SEQ ID NO: 124) was found to be expressed in all head and neck squamous cell tumors tested (17/17): highly expressed in 14/17, and moderately expressed in 3/17. Additionally, expression in lung squamous tumors showed high expression in 3/12 and moderate in 4/12. Contig 11 was negative for 3/4 normal lung samples, with the remaining sample having only low expression. Contig 11 showed low to moderate reactivity to salivary gland, soft palate, bladder, tonsil, skin, esophagus, and large intestine. Contig 13 (SEQ ID NO: 125) was found to be expressed in all head and neck squamous cell tumors tested (17/17): highly expressed in 12/17, and moderately expressed in 5/17. Contig 13 was expressed in 7/12 lung squamous tumors, with high expression in 4/12 and moderate expression in three samples. Analysis of normal lung samples showed negative expression for 2/4 and low to moderate expression in the remaining two samples. Contig 13 did show low to moderate reactivity to resting PBMC, salivary gland, bladder, pancreas, tonsil, skin, esophagus, and large intestine, as well as high expression in soft palate. Contig 16 (SEQ ID NO: 127) was found to be moderately expressed in some head and neck squamous cell tumors (6/17) and one lung squamous tumor; while showing no expression in any normal lung samples tested. Contig 16 did show low reactivity to resting PBMC, large intestine, skin, salivary gland, and soft palate. Contig 17 (SEQ ID NO: 128) was shown to be expressed in all head and neck squamous cell tumors tested (17/17): highly expressed in 5/17, and moderately expressed in 12/17. Expression levels in lung squamous tumors showed one tumor sample with high expression and 3/12 with moderate levels. Contig 17 was negative for 2/4 normal lung samples, with the remaining samples having only low expression. Additionally, low level expression was found in esophagus and soft palate. Contig 19 (SEQ ID NO: 129) was found to be expressed in most head and neck squamous cell tumors tested (11/17): with two samples having high levels, 6/17 showing moderate expression, and low expression being found in 3/17. Testing in lung squamous tumors revealed only moderate expression in 3/12 samples. Expression levels in 2/4 of normal lung samples were negative, the two other samples having only low expression. Contig 19 did show low expression levels in esophagus, resting PBMC, salivary gland, bladder, soft palate, and pancreas.

Contig 22, (SEQ ID NO: 131) was shown to be expressed in most head and neck squamous cell tumors tested (13/17) with high expression in four of these samples, moderate expression in 6/17, and low expression in 3/17. Expression levels in lung squamous tumors were found to be moderate to high for 3/12 tissues tested, with negative expression in two normal lung samples and low expression in two other samples (n=4). Contig 22 did show low expression in skin, salivary gland and soft palate. Similarly, Contig 24 (SEQ ID NO: 132) was found to be expressed in most head and neck squamous cell tumors tested (13/17) with high expression in three of these samples, moderate expression in 6/17, and low expression in 4/17. Expression levels in lung squamous tumors were found to be moderate to high for 3/12 tissues tested, with negative expression for three normal lung samples and low expression in one sample (n=4). Contig 24 did show low expression in skin, salivary gland and soft palate. Contig 29 (SEQ ID NO: 133) was expressed in nearly all head and neck squamous cell tumors tested (16/17): highly expressed in 4/17, moderately expressed in 11/17, with low expression in one sample. Also, it was moderately expressed in 3/12 lung squamous tumors, while being negative for 2/4 normal lung samples. Contig 29 showed low to moderate expression in large intestine, skin, salivary gland, pancreas, tonsil, heart and soft palate. Contig 47 (SEQ ID NO: 142) was expressed in most head and neck squamous cell tumors tested (12/17): moderate expression in 10/17, and low expression in two samples. In lung squamous tumors, it was highly expressed in one sample and moderately expressed in two others (n=13). Contig 47 was negative for 2/4 normal lung samples, with the remaining two samples having moderate expression. Also, Contig 47 showed moderate expression in large intestine, and pancreas, and low expression in skin, salivary gland, soft palate, stomach, bladder, resting PBMC, and tonsil.

Contig 48 (SEQ ID NO: 143) was expressed in all head and neck squamous cell tumors tested (17/17): highly expressed in 8/17 and moderately expressed in 7/17, with low expression in two samples. Expression levels in lung squamous tumors were high to moderate in three samples (n=13). Contig 48 was negative for one out of four normal lung samples, the remaining showing low or moderate expression. Contig 48 showed moderate expression in soft palate, large intestine, pancreas, and bladder, and low expression in esophagus, salivary gland, resting PBMC, and heart. Contig 49 (SEQ ID NO: 144) was

expressed at low to moderate levels in 6/17 head and neck squamous cell tumors tested. Expression levels in lung squamous tumors were moderate in three samples (n=13). Contig 49 was negative for 2/4 normal lung samples, the remaining samples showing low expression. Moderate expression levels in skin, salivary gland, large intestine, pancreas, bladder and resting PBMC were shown, as well as low expression in soft palate, lymph nodes, and tonsil. Contig 56 (SEQ ID NO: 148) was expressed in low to moderate levels in 3/17 head and neck squamous cell tumors tested, and in lung squamous tumors, showing low to moderate levels in three out of thirteen samples. Notably, low expression levels were detected in one adenocarcinoma lung tumor sample (n=2). Contig 56 was negative for 3/4 normal lung samples, and showed moderate expression levels in only large intestine, and low expression in salivary gland, soft palate, pancreas, bladder, and resting PBMC. Contig 58, also known as L769P, (SEQ ID NO: 150) was expressed at moderate levels in 11/17 head and neck squamous cell tumors tested and low expression in one additional sample. Expression in lung squamous tumors showed low to moderate levels in three out of thirteen samples. Contig 58 was negative for 3/4 normal lung samples, with one sample having low expression. Moderate expression levels in skin, large intestine, and resting PBMC were demonstrated, as well as low expression in salivary gland, soft palate, pancreas, and bladder. Contig 59 (SEQ ID NO: 157) was expressed in some head, neck, and lung squamous tumors. Low level expression of Contig 59 was also detected in salivary gland and large intestine.

Additionally, the full-length cDNA sequence for Contigs 22, referred to as L763P, is provided in SEQ ID NO: 158, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 159. Also, the full-length cDNA sequence incorporating Contigs 17, 19, and 24, referred to as L762P, is provided in SEQ ID NO: 160, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 161. Further analysis of L762P has determined it to be a type I membrane protein and two additional variants have been sequenced. Variant 1 (SEQ ID NO: 167 and the corresponding amino acid sequence in SEQ ID NO: 169) is an alternatively spliced form of SEQ ID NO: 160 resulting in deletion of 503 nucleotides, as well as deletion of a short segment of the expressed protein. Variant 2 (SEQ ID NO: 168 and the corresponding amino acid sequence

in SEQ ID NO: 170) has a two nucleotide deletion at the 3' coding region in comparison to SEQ ID NO: 160, resulting in a secreted form of the expressed protein.

The full-length cDNA sequence for contig 56 (SEQ ID NO: 148), referred to as L773P, is provided in SEQ ID NO: 171, with the predicted amino acid sequence in SEQ ID NO: 172. Subsequent Northern blot analysis of L773P demonstrates this transcript is differentially over-expressed in squamous tumors and detected at approximately 1.6 Kb in primary lung tumor tissue and approximately 1.3 Kb in primary head and neck tumor tissue.

Subsequent microarray analysis has shown Contig 58, also referred to as L769S (SEQ ID NO: 150), to be overexpressed in breast tumors in addition to lung squamous tumors.

Example 4
SYNTHESIS OF POLYPEPTIDES

Polypeptides may be synthesized on a Perkin Elmer/Applied Biosystems Division 430A peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

CLAIMS:

1. An isolated polynucleotide molecule comprising a nucleotide sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30, 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157, 158, 160, 167, 168 and 171;

(b) the complements of sequences provided in SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30, 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157, 158, 160, 167, 168 and 171; and

(c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

2. An isolated polypeptide comprising an immunogenic portion of a lung tumor protein or a variant thereof, wherein said protein comprises an amino acid sequence encoded by a polynucleotide molecule of claim 1.

3. An isolated polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide of claim 2.

4. An expression vector comprising an isolated polynucleotide molecule of claims 1 or 3.

5. A host cell transformed with the expression vector of claim 4.

6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cell lines.

7. A pharmaceutical composition comprising the polypeptide of claim 2 and a physiologically acceptable carrier.

8. A vaccine comprising the polypeptide of claim 2 and a non-specific immune response enhancer.

9. The vaccine of claim 8 wherein the non-specific immune response enhancer is an adjuvant.

10. A vaccine comprising an isolated polynucleotide molecule of claims 1 or 3 and a non-specific immune response enhancer.

11. The vaccine of claim 10 wherein the non-specific immune response enhancer is an adjuvant.

12. A pharmaceutical composition for the treatment of lung cancer comprising a polypeptide and a physiologically acceptable carrier, the polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, wherein said protein comprises an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85 , 97-106, 115-124, 126, 130, 134-141, 143, 145-147 and 162-164;

(b) sequences complementary to the sequences of SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85, 97-106, 115-124, 126, 130, 134-141, 143, 145-147 and 162-164 ; and

(c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

13. A vaccine for the treatment of lung cancer comprising a polypeptide and a non-specific immune response enhancer, said polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, wherein said protein comprises an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85, 97-106, 115-124, 126, 130, 134-141, 143, 145-147 and 162-164;

(b) sequences complementary to the sequences of SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85, 97-106, 115-124, 126, 130, 134-141, 143, 145-147 and 162-164; and

(c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

14. A vaccine for the treatment of lung cancer comprising a DNA molecule and a non-specific immune response enhancer, the polynucleotide molecule comprising a sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85, 97-106, 115-124, 126, 130, 134-141, 143, 145-147 and 162-164;

(b) sequences complementary to the sequences of SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85, 97-106, 115-124, 126, 130, 134-141, 143, 145-147 and 162-164; and

(c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

15. A method for inhibiting the development of lung cancer in a patient, comprising administering to the patient an effective amount of the pharmaceutical composition of claims 7 or 12.

16. A method for inhibiting the development of lung cancer in a patient, comprising administering to the patient an effective amount of the vaccine of any one of claims 8, 10, 13 or 14.

17. A fusion protein comprising at least one polypeptide according to claim 2.

18. A fusion protein comprising a polypeptide according to claim 2 and a known lung tumor antigen.

19. A pharmaceutical composition comprising a fusion protein according to any one of claims 17-18 and a physiologically acceptable carrier.

20. A vaccine comprising a fusion protein according to any one of claims 17-18 and a non-specific immune response enhancer.

21. The vaccine of claim 20 wherein the non-specific immune response enhancer is an adjuvant.

22. A method for inhibiting the development of lung cancer in a patient, comprising administering to the patient an effective amount of the pharmaceutical composition of claim 19.

23. A method for inhibiting the development of lung cancer in a patient, comprising administering to the patient an effective amount of the vaccine of claim 20.

24. A method for detecting lung cancer in a patient, comprising:

(a) contacting a biological sample obtained from the patient with a binding agent which is capable of binding to a polypeptide, the polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, wherein said protein comprises an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected

from the group consisting of nucleotide sequences recited in SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171 the complements of said nucleotide sequences and sequences that hybridize to a sequence of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171 under moderately stringent conditions; and

(b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting lung cancer in the patient.

25. The method of claim 24 wherein the binding agent is a monoclonal antibody.

26. The method of claim 25 wherein the binding agent is a polyclonal antibody.

27. A method for monitoring the progression of lung cancer in a patient, comprising:

(a) contacting a biological sample obtained from the patient with a binding agent that is capable of binding to a polypeptide, said polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, wherein said protein comprises an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of nucleotide sequences recited in SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171 the complements of said nucleotide sequences and sequences that hybridize to a nucleotide sequence of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171 under moderately stringent conditions;

(b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b); and

(d) comparing the amount of polypeptide detected in steps (b) and (c) to monitor the progression of lung cancer in the patient.

28. A monoclonal antibody that binds to a polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, wherein said protein comprises an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of: nucleotide sequences recited in SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30, 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157. 158, 160, 167, 168 and 171; the complements of said nucleotide sequences; and sequences that hybridize to a nucleotide sequence of SEQ ID NO: 1-3, 6-8, 10-13, 15-27, 29, 30. 32, 34-49, 51, 52, 54, 55, 57-59, 61-69, 71, 73, 74, 77, 78, 80-82, 84, 86-96, 107-109, 111, 113, 125, 127, 128, 129, 131-133, 142, 144, 148-151, 153, 154, 157, 158, 160, 167, 168 or 171 under moderately stringent conditions.

29. A method for inhibiting the development of lung cancer in a patient, comprising administering to the patient a therapeutically effective amount of a monoclonal antibody according to claim 28.

30. The method of claim 29 wherein the monoclonal antibody is conjugated to a therapeutic agent.

31. A method for detecting lung cancer in a patient comprising:

(a) obtaining a biological sample from the patient;

(b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, wherein at least one of the oligonucleotides is specific for a polynucleotide molecule encoding a polypeptide comprising an immunogenic portion of a lung protein or of a variant thereof, said protein comprising an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of nucleotide sequences recited in SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171 the complements of said nucleotide sequences, and sequences that hybridize to a sequence of SEQ ID NO: 1-109, 111, 113, 115-151. 153, 154, 157, 158, 160, 162-164, 167, 168 or 171 under moderately stringent conditions; and

(c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers, thereby detecting lung cancer.

32. The method of claim 31, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a polynucleotide molecule comprising a sequence selected from SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171.

33. A diagnostic kit comprising:

(a) one or more monoclonal antibodies of claim 28; and

(b) a detection reagent.

34. A diagnostic kit comprising:

(a) one or more monoclonal antibodies that bind to a polypeptide encoded by a polynucleotide molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85, 97-106, 115-124, 126, 130, 134-141, 143, 145-147 and 162-164 the complements of said sequences, and sequences that hybridize to a sequence of SEQ ID NO: 4, 5, 9, 14, 28, 31, 33, 50, 53, 56, 60, 70, 72, 75, 76, 79, 83, 85, 97-106, 115-124, 126, 130, 134-141, 143, 145-147 or 162-164 under moderately stringent conditions; and

(b) a detection reagent.

35. The kit of claims 33 or 34 wherein the monoclonal antibodies are immobilized on a solid support.

36. The kit of claim 35 wherein the solid support comprises nitrocellulose, latex or a plastic material.

37. The kit of claims 33 or 34 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

38. The kit of claim 37 wherein the binding agent is selected from the group consisting of anti-immunoglobulins, Protein G, Protein A and lectins.

39. The kit of claim 37 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

40. A diagnostic kit comprising at least two oligonucleotide primers, at least one of the oligonucleotide primers being specific for a polynucleotide molecule encoding a polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, said protein comprising an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of nucleotide sequences recited in SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171 the complements of said nucleotide sequences and sequences that hybridize to a sequence of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164. 167, 168 or 171 under moderately stringent conditions.

41. A diagnostic kit of claim 40 wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a polynucleotide molecule comprising a sequence selected from SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171.

42. A method for detecting lung cancer in a patient, comprising:

(a) obtaining a biological sample from the patient;

(b) contacting the biological sample with an oligonucleotide probe specific for a polynucleotide molecule encoding a polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, said protein comprising an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of nucleotide sequences recited in SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171 the complements of said nucleotide sequences, and

sequences that hybridize to a sequence of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 or 171 under moderately stringent conditions; and

(c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe, thereby detecting lung cancer in the patient.

43. The method of claim 42 wherein the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide molecule comprising a sequence selected from the group consisting of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171.

44. A diagnostic kit comprising an oligonucleotide probe specific for a polynucleotide molecule encoding a polypeptide comprising an immunogenic portion of a lung protein or a variant thereof, said protein comprising an amino acid sequence encoded by a polynucleotide molecule comprising a sequence selected from the group consisting of: nucleotide sequences recited in SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171; the complements of said nucleotide sequences; and sequences that hybridize to a sequence of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 or 171 under moderately stringent conditions.

45. The diagnostic kit of claim 44, wherein the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide molecule comprising a sequence selected from the group consisting of SEQ ID NO: 1-109, 111, 113, 115-151, 153, 154, 157, 158, 160, 162-164, 167, 168 and 171.

46. A method for treating lung cancer in a patient, comprising the steps of:

(a) obtaining peripheral blood cells from the patient;

(b) incubating the cells in the presence of at least one polypeptide of claim 2, such that T cells proliferate; and

(c) administering to the patient the proliferated T cells.

47. A method for treating lung cancer in a patient, comprising the steps of:

(a) obtaining peripheral blood cells from the patient;

(b) incubating the cells in the presence of at least one polynucleotide of claim 1, such that T cells proliferate; and

(c) administering to the patient the proliferated T cells.

48. The method of any one of claims 46 and 47 wherein the step of incubating the T cells is repeated one or more times.

49. The method of any one of claims 46 and 47 wherein step (a) further comprises separating T cells from the peripheral blood cells, and the cells incubated in step (b) are the T cells.

50. The method of any one of claims 46 and 47 wherein step (a) further comprises separating CD4+ cells or CD8+ cells from the peripheral blood cells, and the cells proliferated in step (b) are CD4+ or CD8+ T cells.

51. The method of any one of claims 46 and 47 wherein step (b) further comprises cloning one or more T cells that proliferated in the presence of the polypeptide.

52. A composition for the treatment of lung cancer in a patient, comprising T cells proliferated in the presence of a polypeptide of claim 2, in combination with a pharmaceutically acceptable carrier.

53. A composition for the treatment of lung cancer in a patient, comprising T cells proliferated in the presence of a polynucleotide of claim 1, in combination with a pharmaceutically acceptable carrier.

54. A method for treating lung cancer in a patient, comprising the steps of:

(a) incubating antigen presenting cells in the presence of at least one polypeptide of claim 2;

(b) administering to the patient the incubated antigen presenting cells.

55. A method for treating lung cancer in a patient, comprising the steps of:

(a) incubating antigen presenting cells in the presence of at least one polynucleotide of claim 1;

(b) administering to the patient the incubated antigen presenting cells.

56. The method of claims 54 or 55 wherein the antigen presenting cells are selected from the group consisting of dendritic cells and macrophage cells.

57. A composition for the treatment of lung cancer in a patient, comprising antigen presenting cells incubated in the presence of a polypeptide of claim 2, in combination with a pharmaceutically acceptable carrier.

58. A composition for the treatment if lung cancer in a patient, comprising antigen presenting cells incubated in the presence of a polynucleotide of claim 1, in combination with a pharmaceutically acceptable carrier.

SEQUENCE LISTING

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caatacacac tcatgaactc ctgatggaac aataacaggc ccaagcctgt ggtatgatgt 240
gcacacttgc tagactcaga aaaaatacta ctctcataaa tgggtgggag tattttgggt 300
gacaacctac tttgcttggc tgagtgaagg aatgatattc atatnttcat ttattccatg 360
gacatttagt tagtgctttt tatataccag gcatgatgct gagtgacact cttgtgtata 420
tntccaaatn ttngtncngt cgctgcacat atctgaaatc ctatattaag antttcccaa 480
natgangtcc ctggtttttc cacgccactt gatcngtcaa ngatcttacc tctgtntgtc 540
ctaaaaccnt ctnctnnang gttagacngg acctctcttc tccctttccg aanaatnaag 600
tgtgngaaga nanccncncn cccccctncn tncnnncctng ccngctnnnc cncntgtngg 660

gggngccgcc cccgcggggg gacccccccn ttttcccc 698

<210> 6
<211> 740
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(740)
<223> n = A,T,C or G

<400> 6
actagtcaaa aatgctaaaa taatttggga gaaaatattt tttaagtagt gttatagttt 60
catgttttatc ttttattatg tnttgtgaag ttgtgtcttt tcactaatta cctatactat 120
gccaatattt ccttatatct atccataaca tttatactac atttgtaaga gaatatgcac 180
gtgaaacttа acactttata aggtaaaaat gaggtttcca agatttaata atctgatcaa 240
gttcttgtta tttccaaata gaatggactt ggtctgttaa ggggctaagg gagaagaaga 300
agataaggtt aaaagttgtt aatgaccaaa cattctaaaa gaaatgcaaa aaaaaattta 360
ttttcaagcc ttcgaactat ttaaggaaag caaaatcatt tcctanatgc atatcatttg 420
tgaganttte tcantaatat cctgaatcat tcatttcagc tnaggcttca tgttgactcg 480
atatgtcatc tagggaaagt ctatttcatg gtccaaacct gttgccatag ttggtnaggc 540
ttttcttttaa ntgtgaanta ttnacangaa attttctctt tnanagttct tnatagggtt 600
aggggtgtgg gaaaagcttt taacaatctg tagtgttncg tgttatctgt ncagaaccan 660
aatnacggat cgnangaagg actgggtcta tttacangaa cgaatnatct ngttnnntgt 720
gtnnncaact ccngggagcc 740

<210> 7
<211> 670
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(670)
<223> n = A,T,C or G

<400> 7
gctggggagc tcggcatggc ggtccccgct gcagccatgg ggccctcggc gttgggccag 60
agcggccccg gctcgatggc cccgtggtgc tcagtgagca gcggccccgtc gcgctacgtg 120
cttgggatgc aggagctgtt ccggggccac agcaagaccg cgagttcctg gcgcacagcg 180
ccaaggtgca ctcggtggcc tggagttgcg acgggcgtcg cctacctcgg ggtcttcgac 240
aagacgccac gtcttcttgc tgganaanga ccgttggtca aagaaaacaa ttatcgggga 300
catggggata gtgtggacca ctttgttggc atccaagtaa tcctgaccta tttgttacgg 360
cgtctggaga taaaaccatt cgcatctggg atgtgaggac tacaaaatgc attgccactg 420
tgaacactaa aggggagaac attaatatct gctggantcc tgatgggcan accattgctg 480
tagcnacaag gatgatgtgg tgacttttatt gatgccaaga aaccccgttc caaagcaaaa 540
aaacanttcc aanttcgaag tcaccnaaat ctcctggaac aatgaacatn aatatnttct 600
tcctgacaat ggnccttggg tgtntcacat cctcagctnc cccaaaactg aancctgtnc 660
natccacccc 670

<210> 8
<211> 689
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(689)
<223> n = A,T,C or G

<400> 8
actagtatct aggaatgaac agtaaaagag gagcagttgg ctacttgatt acaacagagt 60
aaatgaagta ctggatttgg gaaaacctgg ttttattaga acatatggaa tgaaagccta 120
cacctagcat tgcctactta gccccctgaa ttaacagagc ccaattgaga caaaccccctg 180
gcaacaggaa attcaaggga gaaaaagtaa gcaacttggg ctaggatgag ctgactcccc 240
tagagcaaag ganagacagc ccccattacc aaataccatt tttgcctggg gcttgtgcag 300
ctggcagtgt tcctgcccca gcatggcacc ttatngtttt gatagcaact tcgttgaatt 360
ttcaccaact tattacttga aattataata tagcctgtcc gtttgctgtn tccaggctgt 420
gatatatntt cctagtggtt tgactttnaa aataaatnag gtttcantttt ctccccccnn 480
cnntnctncc nntcnctcnn cnntcccccc cnctcngtcc tccnnnnttn ggggggccn 540
ccccccnggn ggaccccccc ttggtcccct agtggaggtt natggccccc ggnnttatcc 600
nggccntann tttccccgtn nnaaatgntt ccccctccca ntcccncccac ctccaancccgg 660
aagcctaagt ttntaccctg ggggtcccc 689

<210> 9
<211> 674
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(674)
<223> n = A,T,C or G

<400> 9
gtccactctc ctttgagtgt actgtcttac tgtgcactct gtttttcaac tttctagata 60
taaaaaatgc ttgttctata gtggagtaag agctcacaca cccaaggcag caagataact 120
gaaaaaagcg aggctttttt gccaccttgg taaaggccag ttcactgcta tagaactgct 180
ataagcctga agggaagtag ctatgagact ttccattttt cttagttctc ccaataggct 240
ccttcatgga aaaaggcttc ctgtaataat tttcacctaa tgaattagca gtgtgattac 300
ttctgaaata agagacaaat tgggccgcag agtcttcctg tgatttaaaa taaacaaccc 360
aaagttttgt ttggtcttca ccaaaggaca tactctaggg ggtatgttgt tgaagacatt 420
caaaaacatt agctgttctg tctttcaatt tcaagttatt ttggagactg cctccatgtg 480
agttaattac tttgctctgg aactagcatt attgtcatta tcatcacatt ctgtcatcat 540
catctgaata atattgtgga tttccccctc tgcttgcatc ttcttttgac tcctctggga 600
anaaatgtca aaaaaaaagg tcgatctact cngcaaggnc catctaatca ctgcgctgga 660
aggacccnct gccc 674

<210> 10
<211> 346
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(346)
<223> n = A,T,C or G

<400> 10

<221> misc_feature
<222> (1)...(694)
<223> n = A,T,C or G

<400> 13
cactagtcac tcattagcgt tttcaatagg gctcttaagt ccagtagatt acgggtagtc 60
agttgacgaa gatctggttt acaagaacta attaaatgtt tcattgcatt tttgtaagaa 120
cagaataatt ttataaaatg tttgtagttt ataattgccg aaaataattt aaagacactt 180
tttctctgtg tgtgcaaatg tgtgtttgtg atccattttt ttttttttts taggacacct 240
gtttactagc tagctttaca atatgccaaa aaaggatttc tccctgaccc catccgtggt 300
tcaccctctt ttccccccat gcttttgcc ctagtttata acaaaggaat gatgatgatt 360
taaaaagtag ttctgtatct tcagtatctt ggtcttccag aaccctctgg ttgggaaggg 420
gatcattttt tactggtcat ttccctttgg agtgtactac tttaacagat ggaaagaact 480
cattggccat ggaaacagcc gangtgttgg gagccagcag tgcatggcac cgtccggcat 540
ctggcntgat tggtctggct gccgtcattg tcagcacagt gccatgggac atggggaana 600
ctgactgcac ngccaatggt tttcatgaag aatacngcat ncncngtgat cacgtnancc 660
angacgctat gggggncana gggccanttg cttc 694

<210> 14
<211> 679
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(679)
<223> n = A,T,C or G

<400> 14
cagccgcctg catctgtatc cagcgccang tcccgccagt cccagctgcg cgcgcccccc 60
agtcccgnac ccgttcggcc cangctnagt tagncctcac catnccggtc aaaggangca 120
ccaagtgcat caaatacctg cngtncggat ntaaattcat cttctggctt gccgggattg 180
ctgtccntgc cattggacta nggctccgat ncgactctca gaccanganc atcttcganc 240
naganactaa tnatnattnt tccagcttct acacaggagt ctatattctg atcggatccg 300
gcnccctcnt gatgctggtg ggcttcctga gctgctgcgg ggctgtgcaa gagtcccant 360
gcatgctggg actgttcttc ggcttcntct tggtgatatn cgccattgaa atacctgcgg 420
ccatctgggg atattccact ncgatnatgt gattaaggaa ntccacggag ttttacaagg 480
acacgtacaa cnacctgaaa accnnggatg ancccaccg ggaancnctg aangccatcc 540
actatgcgtt gaactgcaat ggtttggctg gggnccttga acaatttaat cncatacatc 600
tggccccann aaaggacntn ctcganncct tcnccgtgna attcngttct gatnccatca 660
cagaagtctc gaacaatcc 679

<210> 15
<211> 695
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(695)
<223> n = A,T,C or G

<400> 15
actagtggat aaaggccagg gatgctgctc aacctcctac catgtacagg gacgtcccc 60
cattacaact accaaatccg aagtgtcaac tgtgtcagga ctaanaaacc ctggttttga 120

actagtctgc tgatagaaag cactatacat cctattgttt ctttctttcc aaaatcagcc 60
ttctgtctgt aacaaaaatg tactttatag agatggagga aaaggtctaa tactacatag 120
ccttaagtgt ttctgtcatt gttcaagtgt attttctgta acagaaacat atttggaatg 180
ttttttcttt ccccttataa attgtaattc ctgaaatact gctgctttaa aaagtcccac 240
tgtcagatta tattatctaa caattgaata ttgtaaatat acttgtctta cctcttcaata 300
aaagggtact tttctattan nnagnngnnn gnnnnataaa anaaaa 346

<210> 11
<211> 602
<212> DNA
<213> Homo sapien

<400> 11
actagtaaaa agcagcattg ccaaataatc cctaatttta cactaaaaat ataatgaaat 60
gatgttaagc tttttgaaaa gtttaggtta aacctactgt tgttagatta atgtatttgt 120
tgcttcccct tatctggaat gtggcattag cttttttatt ttaaccctct ttaattctta 180
ttcaattcca tgacttaagg ttggagagct aaacactggg atttttggat aacagactga 240
cagttttgca taattataat cggcattgta catagaaagg atatggctac cttttgttaa 300
atctgcactt tctaaatatc aaaaaaggga aatgaagtta taaatcaatt tttgtataat 360
ctgtttgaaa catgagtttt atttgcttaa tattagggct ttgccccttt tctgtaagtc 420
tcttgggatc ctgtgtagaa ctgttctcat taaacaccaa acagttaagt ccattctctg 480
gtacttagcta caaattcggt ttcatatttct acttaacaat ttaaataaac tgaaatattt 540
ctagatggtc tacttctgtt catataaaaa caaaacttga tttccaaaaa aaaaaaaaaa 600
aa 602

<210> 12
<211> 685
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(685)
<223> n = A,T,C or G

<400> 12
actagtcctg tgaaagtaca actgaaggca gaaagtgtta ggattttgca tctaatgttc 60
attatcatgg tattgatgga cctaagaaaa taaaaattag actaagcccc caaataagct 120
gcatgcattt gtaacatgat tagtagattt gaatatatag atgtagtatn ttgggtatct 180
aggtgtttta tcattatgta aaggaattaa agtaaaggac tttgtagttg tttttattaa 240
atatgcatat agtagagtgc aaaaatatag caaaaatana aactaaaggt agaaaagcat 300
tttagatatg ccttaatnta nnaactgtgc caggtggccc tcggaataga tgccaggcag 360
agaccagtgc ctgggtggtg cctccccttg tctgcccccc tgaagaactt ccctcacgtg 420
angtagtgcc ctcgtaggtg tcacgtggan tantggganc aggccgnncn gtnanaagaa 480
ancanngtga nagtttcncc gtngangcng aactgtccct gngccnnnac gctcccanaa 540
cntnttccaat ngacaatcga gtttccnnnc tccngnaacc tngccgnnnn cnngcccnnc 600
cantntgnta acccccgcgcc cggatcgctc tcnnntcgtt ctcncncnaa ngggnttttcn 660
cnnccgccgt cncnnccccg cnncc 685

<210> 13
<211> 694
<212> DNA
<213> Homo sapien

<220>

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ttaaaaaagg gcctgaaaaa aggggagcca caaatctgtc tgcttcctca cnttantcnt   180
tggcaaatna gcattctgtc tcnttggctg cngcctcanc ncaaaaaanc ngaactcnat   240
cnggcccagg aatacatctc ncaatnaacn aaattganca aggcnntggg aaatgccnga   300
tgggattatc ntccgcttgt tgancttcta agtttcnttc cttcatten accctgccag   360
ccnagttctg ttagaaaaat gccngaattc naacnccggt tttcntactc ngaatttaga   420
tctncanaaa cttcctggcc acnattcnaa ttnanggnca cgnacanatn ccttccatna   480
ancncaccac acntttgana gccangacaa tgactgcntn aantgaaggc ntgaaggaan   540
aacttttgaaa ggaaaaaaaa ctttgtttcc ggcccccttcc aacnctttctg tgttnancac   600
tgccttctng naaccctgga agcccngnga cagtgttaca tgttgttcta nnaaacngac   660
ncttnaatnt cnatcttccc nanaacgatt ncncc                               695
```

<210> 16
<211> 669
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(669)
<223> n = A,T,C or G

<400> 16

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cgccgaagca gcagcgcagg ttgtcccccgt ttcccctccc ccttcccttc tccggttgcc    60
ttcccgggcc ccttacactc cacagtcccg gtcccgccat gtcccagaaa caagaagaag   120
agaaccctgc ggaggagacc ggcgaggaga agcaggacac gcaggagaaa gaaggtattc   180
tgcctgagag agctgaagag gcaaagctaa aggccaaata cccaagccta ggacaaaagc   240
ctggaggctc cgacttcctc atgaagagac tccagaaagg gcaaaagtac tttgactcng   300
gagactacaa catggccaaa gccaacatga agaataagca gctgccaagt gcangaccag   360
acaagaaccc ggtgactggt gatcacatcc ccacccccaca ggatctgccc agagaaagtc   420
ctcgctcgtc accagcaagc ttgcgggtgg ccaagttgaa tgatgctgcc ggggctctgc   480
canatctgag acgcttcccc cccctgcccca cccgggtcct gtgctggctc ctgccccttcc   540
tgcttttgca gccangggtc aggaagtggc ncnggtngtg gctggaaagc aaaaccctttt   600
cctgttggtg tcccacccat ggagcccctg gggcgagccc angaacttga ncctttttgt   660
tntcttncc                                                           669
```

<210> 17
<211> 697
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(697)
<223> n = A,T,C or G

<400> 17

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gcaagatatg gacaactaag tgagaaggta atnctctact gctctagntn ctccnggcnn    60
gacgcgctga ggagannnac gctggcccan ctgccggcca cacacgggga tcntggtnat   120
gcctgcccan gggancccca ncnctcggan cccatntcac acccgnnccn tncgcccacn   180
ncctggctcn cncngcccng nccagctcnc gncccccctcc gccnnnnctcn ttnnncntctc   240
cncncccctcc ncnacnacct cctacccncg gctcccctccc cagccccccc ccgcaancct   300
ccacnacncc ntcnncncga ancncccntc gcnctcngcc ccngccccct gccccccgcc   360
cncnacnncg cgntcccccg cgcncgcngc ctcncccccct cccacnacag ncncacccgc   420
agncacgcnc tccgcccnct gacgcccccnn cccgccgcgc tcacctttcat ggncccnacng   480
ccccgctcnc ncccctgcnc gccgncnngg cgccccgccc cnncccgngtn ccncncgnng   540
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ccccngcngn angcngtgcg cnncangncc gngccgnncn ncaccctccg nccnccgccc 600
cgcccgctgg gggctcccgc cncgcggntc antccccncc cntncgccca ctntccgntc 660
cnncnctcnc gctcngcgcn cgcccncccc ccccccc 697

<210> 18
<211> 670
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(670)
<223> n = A,T,C or G

<400> 18
ctcgtgtgaa gggtgcagta cctaagccgg agcggggtag aggcgggccg gcaccccctt 60
ctgacctcca gtgccgccgg cctcaagatc agacatggcc cagaacttga acgacttggc 120
gggacggctg cccgccgggc cccggggcat gggcacggcc ctgaagctgt tgctgggggc 180
cggcgccgtg gcctacggtg tgcgcgaatc tgtgttcacc gtggaaggcg ggcncagagc 240
catcttcttc aatcggatcg gtggagtgca caggacacta tcctgggccg anggccttca 300
cttcaggatc cttggttcca gtaccccanc atctatgaca ttcgggccag acctcgaaaa 360
aatctcctcc ctacaggctc caaagaccta cagatggtga atatctcccc gcgagtgttg 420
tctcgaccaa tgctcangaa cttcctaaca tgttccancg cctaagggct ggactacnaa 480
gaacgantgt tgccgtccat tgtcacgaag tgctcaagaa tttnggtggc caagttcaat 540
gncctcacnn ctgatcnccc agcggggcca agttanccct ggttgatccc cgggganctg 600
acnnaaaagg gccaaggact tccccttcatc ctggataatg tggccntcac aaagctcaac 660
tttanccacc 670

<210> 19
<211> 606
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(606)
<223> n = A,T,C or G

<400> 19
actagtgcca acctcagctc ccaggccagt tctctgaatg tcgaggagtt ccaggatctc 60
tggcctcagt tgtccttggt tattgatggg ggacaaattg gggatggcca gagccccgag 120
tgtcgccttg gctcaactgt ggttgatttg tctgtgcccg gaaagtttgg catcattcgt 180
ccaggctgtg ccctggaaag tactacagcc atcctccaac agaagtacgg actgctcccc 240
tcacatgcgt cctacctgtg aaactctggg aagcaggaag gcccaagacc tggtgctgga 300
tactatgtgt ctgtccactg acgactgtca aggcctcatt tgcagaggcc accggagcta 360
gggcactagc ctgactttta aggcagtgtg tctttctgag cactgtagac caagcccttg 420
gagctgctgg tttagccttg cacctgggga aaggatgtat ttatttgtat tttcatatat 480
cagccaaaag ctgaatggaa aagttnagaa cattcctagg tggccttatt ctaataagtt 540
tctttgtctt gttttgtttt tcaattgaaa agttattaaa taacagattt agaatctagt 600
gagacc 606

<210> 20
<211> 449
<212> DNA
<213> Homo sapien

<400> 20
actagtaaac aacagcagca gaaacatcag tatcagcagc gtcgccagca ggagaatatg 60
cagcgccaga gccgaggaga acccccgctc cctgaggagg acctgtccaa actcttcaaa 120
ccaccacagc cgcctgccag gatggactcg ctgctcattg caggccagat aaacacttac 180
tgccagaaca tcaaggagtt cactgcccaa aacttaggca agctcttcat ggcccaggct 240
cttcaagaat acaacaacta agaaaaggaa gttttccagaa aagaagttaa catgaactct 300
tgaagtcaca ccagggcaac tcttggaaga aatatatttg catattgaaa agcacagagg 360
atttctttag tgtcattgcc gattttggct ataacagtgt ctttctagcc ataataaaat 420
aaaacaaaat cttgactgct tgcttcaaaa 449

<210> 21
<211> 409
<212> DNA
<213> Homo sapien

<400> 21
tatcaatcaa ctggtgaata attaaacaat gtgtggtgtg atcatacaaa gggtaccact 60
caatgataaa aggaacaagc tgcctatatg tggaacaaca tggatgcatt tcagaaactt 120
tatgttgagt gaaagaacaa acacggagaa catactatgt ggttctcttt atgtaacatt 180
acagaaataa aaacagaggc aaccaccttt gaggcagtat ggagtgagat agactggaaa 240
aaggaaggaa ggaaactcta cgctgatgga aatgtctgtg tcttcattgg gtggtagtta 300
tgtggggata tacacttgtc aaaatttatt gaactatata ctaaagaact ctgcatttta 360
ttgggatgta aataatacct caattaaaaa gacaaaaaaa aaaaaaaaa 409

<210> 22
<211> 649
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(649)
<223> n = A,T,C or G

<400> 22
acaattttca ttatcttaag cacattgtac atttctacag aacctgtgat tattctgca 60
tgataaggat ggtacttgca tatggtgaat tactactgtt gacagtttcc gcagaaatcc 120
tatttcagtg gaccaacatt gtggcatggc agcaaatgcc aacattttgt ggaatagcag 180
caaatctaca agagaccctg gttggttttt cgttttgttt tctttgtttt ttccccttc 240
tcctgaatca gcagggatgg aangagggta gggaagttat gaattactcc ttccagtagt 300
agctctgaag tgtcacattt aatatcagtt ttttttaaac atgattctag ttnaatgtag 360
aagagagaag aaagaggaag tgttcacttt tttaatacac tgatttagaa atttgatgtc 420
ttatatcagt agttctgagg tattgatagc ttgctttatt tctgccttta cgttgacagt 480
gttgaagcag ggtgaataac taggggcata tatatttttt ttttttgtaa gctgtttcat 540
gatgttttct ttggaatttc cggataagtt caggaaaaca tctgcatgtt gttatctagt 600
ctgaagttcn tatccatctc attacaacaa aaacncccag aacggnttg 649

<210> 23
<211> 669
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature

<222> (1)...(669)
<223> n = A,T,C or G

<400> 23
actagtgccg tactggctga aatccctgca ggaccaggaa gagaaccagt tcagactttg 60
tactctcagt caccagctct ggaattagat aaattccttg aagatgtcag gaatgggatc 120
tatcctctga cagcctttgg gctgcctcgg ccccagcagc cacagcagga ggaggtgaca 180
tcacctgtcg tgccccccta tgtcaagact ccgacacctg aaccagctga ggtggagact 240
cgcaaggtgg tgctgatgca gtgcaacatt gagtcggtgg aggagggagt caaacaccac 300
ctgacacttc tgctgaagtt ggaggacaaa ctgaaccggc acctgagctg tgacctgatg 360
ccaaatgaga atatccccga gttggcggct gagctggtgc agctgggctt cattagtgag 420
gctgaccaga gccggttgac ttctctgcta gaagagactt gaacaagttc aattttgcca 480
ggaacagtac cctcaactca gccgctgtca ccgtctcctc ttagagctca ctcgggccag 540
gccctgatct gcgctgtggc tgtcctggac gtgctgcacc ctctgtcctt ccccccagtc 600
agtattacct gtgaagccct tccctccttt attattcagg anggctgggg gggctccttg 660
nttctaacc 669

<210> 24
<211> 442
<212> DNA
<213> Homo sapien

<400> 24
actagtacca tcttgacaga ggatacatgc tcccaaaacg tttgttacca cacttaaaaa 60
tcactgccat cattaagcat cagtttcaaa attatagcca ttcatgattt actttttcca 120
gatgactatc attattctag tcctttgaat ttgtaagggg aaaaaaaaca aaaacaaaaa 180
cttacgatgc actttttctc agcacatcag atttcaaatt gaaaattaaa gacatgctat 240
ggtaatgcac ttgctagtac tacacacttt ggtacaacaa aaaacagagg caagaaacaa 300
cggaaagaga aaagccttcc tttgttggcc cttaaactga gtcaagatct gaaatgtaga 360
gatgatctct gacgatacct gtatgttctt attgtgtaaa taaaattgct ggtatgaaat 420
gacctaaaaa aaaaaaaaga aa 442

<210> 25
<211> 656
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(656)
<223> n = A,T,C or G

<400> 25
tgcaagtacc acacactgtt tgaattttgc acaaaaagtg actgtaggat caggtgatag 60
ccccggaatg tacagtgtct tggtgcacca agatgccttc taaaggctga cataccttgg 120
accctaatgg ggcagagagt atagccctag cccagtggtg acatgaccac tccctttggg 180
aggcctgagg tagaggggag tggtatgtgt tttctcagtg gaagcagcac atgagtgggt 240
gacaggatgt tagataaagg ctctagttag ggtgtcattg tcatttgaga gactgacaca 300
ctcctagcag ctggtaaagg ggtgctggan gccatggagg anctctagaa acattagcat 360
gggctgatct gattacttcc tggcatcccg ctcacttttа tgggaagtct tattagangg 420
atgggacagt tttccatatc cttgctgtgg agctctggaa cactctctaa atttcccctt 480
attaaaaatc actgccctaa ctacacttcc tccttgaagg aatagaaatg gaacttttcc 540
tgacatantt cttggcatgg ggagccagcc acaaatgana atctgaacgt gtccaggttt 600
ctcctganac tcatctacat agaattggtt aaaccctccc ttggaataag gaaaaa 656

<210> 26
<211> 434
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(434)
<223> n = A,T,C or G

<400> 26
actagttcag actgccacgc caaccccaga aaatacccca catgccagaa aagtgaagtc 60
ctaggtgttt ccatctatgt ttcaatctgt ccatctacca ggcctcgcga taaaaacaaa 120
acaaaaaaac gctgccaggt tttagaagca gttctggtct caaaaccatc aggatcctgc 180
caccagggtt cttttgaaat agtaccacat gtaaaaggga atttggcttt cacttcatct 240
aataactgaa ttgtcaggct ttgattgata attgtagaaa taagtagcct tctgttgtgg 300
gaataagtta taatcagtat tcatctcttt gttttttgtc actcttttct ctctaattgt 360
gtcatttgta ctgtttgaaa aatatttctt ctatnaaatt aaactaacct gccttaaaaa 420
aaaaaaaaaa aaaa 434

<210> 27
<211> 654
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(654)
<223> n = A,T,C or G

<400> 27
actagtccaa cacagtcaga aacattgttt tgaatcctct gtaaaccaag gcattaatct 60
taataaacca ggatccattt aggtaccact tgatataaaa aggatatcca taatgaatat 120
tttatactgc atcctttaca ttagccacta aatacgttat tgcttgatga agacctttca 180
cagaatccta tggattgcag catttcactt ggctacttca tacccatgcc ttaaagaggg 240
gcagtttctc aaaagcagaa acatgccgcc agttctcaag tttttcctct aactccattt 300
gaatgtaagg gcagctggcc cccaatgtgg ggaggtccga acatttttctg aattcccatt 360
ttcttgttcg cggctaaatg acagtttctg tcattactta gattccgatc tttcccaaag 420
gtgttgattt acaaagaggc cagctaatag cagaaatcat gaccctgaaa gagagatgaa 480
attcaagctg tgagccaggc agganctcag tatggcaaag gtcttgagaa tcngccattt 540
ggtacaaaaa aaattttaaa gcntttatgt tataccatgg aaccatagaa anggcaaggg 600
aattgttaag aanaatttta agtgtccaga cccanaanga aaaaaaaaaa aaaa 654

<210> 28
<211> 670
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(670)
<223> n = A,T,C or G

<400> 28
cgtgtgcaca tactgggagg atttccacag ctgcacggtc acagcccctta cggattgcca 60

ggaaggggcg aaagatatgt gggataaact gagaaaagaa nccaaaaacc tcaacatcca 120
aggcagctta ttcgaactct gcggcagcgg caacggggcg gcggggtccc tgctcccggc 180
gttcccggtg ctcctggtgt ctctctcggc agctttagcg acctgncttt cctttgagc 240
gtggggccag ctccccccgc ggcgcccacc cacnctcact ccatgctccc ggaaatcgag 300
aggaagatca ttagttcttt ggggacgttn gtgattctct gtgatgctga aaaacactca 360
tatagggaat gtgggaaatc ctganctctt tnttatntcg tntgatttct tgtgttttat 420
ttgccaaaat gttaccaatc agtgaccaac cnagcacagc caaaaatcgg acntcngctt 480
tagtccgtct tcacacacag aataagaaaa cggcaaaccc accccacttt tnantttnat 540
tattactaan ttttttctgt tgggcaaaag aatctcagga acngccctgg ggccnccgta 600
ctanagttaa ccnagctagt tncatgaaaa atgatgggct ccncctcaat gggaaagcca 660
agaaaaagnc 670

<210> 29
<211> 551
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(551)
<223> n = A,T,C or G

<400> 29
actagtcctc cacagcctgt gaatcccgct agacctttca agcatagtga gcggagaaga 60
agatctcagc gtttagccac cttacccatg cctgatgatt ctgtagaaaa ggtttcttct 120
ccctctccag ccactgatgg gaaagtattc tccatcagtt ctcaaaatca gcaagaatct 180
tcagtaccag aggtgcctga tgttgcacac ttgccacttg agaagctggg accctgtctc 240
cctcttgact taagtcgtgg ttcagaagtt acagcaccgg tagcctcaga ttcctcttac 300
cgtaatgaat gtcccagggc agaaaaagag gatacncaga tgcttccaaa tcctttcttc 360
aaagcaatag ctgatgggaa gaggagctcc agcagcagca ggaatatcga aaacagaaaa 420
aaaagtgaaa ttgggaagac aaaagctcaa cagcatttgg taaggagaaa aganaagatg 480
aggaaggaag agagaagaga gacnaagatc nctacggacc gnnncggaag aagaagaagn 540
aaaaaanaaa a 551

<210> 30
<211> 684
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(684)
<223> n = A,T,C or G

<400> 30
actagttcta tctggaaaaa gcccgggttg gaagaagctg tggagagtgc gtgtgcaatg 60
cgagactcat ttcttggaag catcccctggc aaaaatgcag ctgagtacaa ggttatcact 120
gtgatagaac ctggactgct ttttgagata atagagatgc tgcagtctga agagacttcc 180
agcaccttctc agttgaatga attaatgatg gctttctgagt caacttttact ggctcaggaa 240
ccacgagaga tgactgcaga tgtaatcgag cttaaaggga aattcctcat caacttagaa 300
ggtggtgata ttcgtgaaga gtcttcctat aaagtaattg tcatgccgac tacgaaagaa 360
aaatgccccc gttgttggaa gtatacagcg ggagtcttca gatacactgt gtcctgatg 420
tgcagaagtt gtcagtggga aaatagtatt aacagctcac tcgagcaaga accctcctga 480
cagtactggg ctagaagttt ggatggatta tttacaatat aggaaagaaa gccaagaatt 540
aggtnatgag tggatgagta aatggtggan gatggggaat tcaaatcaga attatggaag 600

aagttnttcc tgttactata gaaaggaatt atgtttattt acatgcagaa aatatanatg 660
tgtggtgtgt accgtggatg gaan 684

<210> 31
<211> 654
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(654)
<223> n = A,T,C or G

<400> 31
gcgcagaaaa ggaaccaata tttcagaaac aagcttaata ggaacagctg cctgtacatc 60
aacatctttt cagaatgacc cagaagttat catcgtggga gctggcgtgc ttggctctgc 120
tttggcagct gtgctttcca gagatggaag aaaggtgaca gtcattgaga gagacttaaa 180
agagcctgac agaatagttg gagaattcct gcagccgggt ggttatcatg ttctcaaaga 240
ccttggtctt ggagatacag tggaaggtct tgatgcccag gttgtaaatg gttacatgat 300
tcatgatcag ggaaagcaaa tcagangttc agattcctta ccctctgtca gaaaacaatc 360
aagtgcagag tggaagagct ttccatcacg gaagattcat catgagtctc cggaaagcag 420
ctatggcaga gcccaatgca aagtttattg aaggtgttgt gttacagtta ttagaggaag 480
atgatgttgt gatgggagtt cagtacaagg ataaagagac tgggagatat caaggaactc 540
catgctccac tgactgttgt tgcagatggg cttttctcca anttcaggaa aagcctggtc 600
tcaataaagt ttctgtatca ctcatttggt tggcttctta tgaagaatgc nccc 654

<210> 32
<211> 673
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(673)
<223> n = A,T,C or G

<400> 32
actagtgaag aaaaagaaat tctgatacgg gacaaaaatg ctcttcaaaa catcattctt 60
tatcacctga caccaggagt tttcattgga aaaggatttg aacctggtgt tactaacatt 120
ttaaagacca cacaaggaag caaaatcttt ctgaaagaag taaatgatac acttctggtg 180
aatgaattga aatcaaaaga atctgacatc atgacaacaa atggtgtaat tcatgttgta 240
gataaactcc tctatccagc agacacacct gttggaaatg atcaactgct ggaaatactt 300
aataaattaa tcaaatacat ccaaattaag tttgttcgtg gtagcacctt caaagaaatc 360
cccgtgactg tctatnagcc aattattaaa aaatacacca aaatcattga tgggagtgcc 420
tgtgggaaat aactgaaaaa gagaccgaga agaacgaatc attacaggtc ctgaaataaa 480
atacctagga tttctactgg aggtggagaa acagaagaac tctgaagaaa ttgttacaag 540
aagangtccc aaggtcacca aattcattga aggtggtgat ggtctttatt tgaagatgaa 600
gaaattaaaa gacgcttcag ggagacnccc catgaaggaa ttgccagcca caaaaaaatt 660
cagggattag aaa 673

<210> 33
<211> 673
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(673)
<223> n = A,T,C or G

<400> 33
actagttatt tactttcctc cgcttcagaa ggtttttcag actgagagcc taagcatact 60
ggatctgttg tttcttttgg gtctcacctc atcagtgtgc atagtggcag aaattataaa 120
gaaggttgaa aggagcaggg aaaagatcca gaagcatgtt agttcgacat catcatcttt 180
tcttgaagta tgatgcatat tgcattattt tatttgcaaa ctaggaattg cagtctgagg 240
atcatttaga agggcaagtt caagaggata tgaagatttg agaacttttt aactattcat 300
tgactaaaaa tgaacattaa tgttnaagac ttaagacttt aacctgctgg cagtcccaaa 360
tgaaattatg caactttgat atcatattcc ttgatttaaa ttgggctttt gtgattgant 420
gaaactttat aaagcatatg gtcagttatt tnattaaaaa ggcaaaaact gaaccacctt 480
ctgcacttaa agaagtctaa cagtacaaat acctatctat cttagatgga tntatttntt 540
tntatttttа aatattgtac tatttatggt nggtggggct ttcttactaa tacacaaatn 600
aatttatcat ttcaanggca ttctatttgg gtttagaagt tgattccaag nantgcatat 660
ttcgctactg tnt 673

<210> 34
<211> 684
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(684)
<223> n = A,T,C or G

<400> 34
actagtttat tcaagaaaag aacttactga ttcctctgtt cctaaagcaa gagtggcagg 60
tgattcagggc tggtgtagca tccggttcct ttagtgcagc taactgcatt tgtcactgat 120
gaccaaggag gaaatcacta agacatttga gaagcagtgg tatgaacgtt cttggacaag 180
ccacagttct gagccttaac cctgtagttt gcacacaaga acgagctcca cctccccttc 240
ttcaggagga atctgtgcgg atagattggc tggacttttc aatggttctg ggttgcaagt 300
gggcactgtt atggctgggt atggagcgga cagccccagg aatcagagcc tcagcccggc 360
tgcctggttg gaaggtacag gtgttcagca ccttcggaaa aagggcataa agtngtgggg 420
gacaattctc agtccaagaa gaatgcattg accattgctg gctatttgct tncctagtan 480
gaattggatn cattttgac cangatnntt ctnctatgct ttnttgcaat gaaatcaaat 540
cccgcattat ctacaagtgg tatgaagtcc tgcnnccccc agagaggctg ttcaggcnat 600
gtcttccaag ggcagggtgg gttacaccat tttacctccc ctctcccccc agattatgna 660
cncagaagga atttntttcc tccc 684

<210> 35
<211> 614
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(614)
<223> n = A,T,C or G

<400> 35
actagtccaa cgcgttngcn aatattcccc tggtagccta cttccttacc cccgaatatt 60

ggtaagatcg agcaatggct tcaggacatg ggttctcttc tcctgtgatc attcaagtgc 120
tcactgcatg aagactggct tgtctcagtg tntcaacctc accagggctg tctcttggtc 180
cacacctcgc tcccctgttag tgccgtatga cagcccccat canatgacct tggccaagtc 240
acggtttctc tgtggtcaat gttggtnggc tgattggtgg aaagtanggt ggaccaaagg 300
aagncncgtg agcagncanc nccagttctg caccagcagc gcctccgtcc tactngggtg 360
ttccngtttc tcctggccct gngtgggcta nggcctgatt cgggaanatg cctttgcang 420
gaaggganga taantgggat ctaccaattg attctggcaa aacnatntct aagattnttn 480
tgctttatgt ggganacana tctanctctc atttnntgct gnanatnaca ccctactcgt 540
gntcgancnc gtcttcgatt ttcgganaca cnccantnaa tactggcgtt ctgttgttaa 600
aaaaaaaaaa aaaa 614

<210> 36
<211> 686
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(686)
<223> n = A,T,C or G

<400> 36
gtggctggcc cggttcttcg cttctcccca tcccctactt tcctcccttc ctcccttccc 60
ctcctcgtc gactgttgct tgctggtcgc agactcctg acccctcccc caccccttcc 120
taacctcggt gccaccggat tgcccttctt ttcctgttgc ccagcccagc cctagtgtca 180
gggcgggggc ctggagcagc ccgaggcact gcagcagaag ananaaaaga cacgacnaac 240
ctcagctcgc cagtccggtc gctngcttcc cgccgcatgg caatnagaca gacgccgctc 300
acctgcttcg ggcacacgcg acccgtggtt gatttggcct tcagtggcat caccctatg 360
ggtatttctt aatcagcgct tgcaaagatg gttaacctat gctacgccag ggagatacag 420
gagactggat tggaacattt ttggggtcta aaggtctgtt tggggtgcaa cactgaataa 480
ggatgccacc aaagcagcta cagcagctgc agatttcaca gcccaagtgt gggatgctgt 540
ctcagganat naattgataa cctggctcat aacacattgt caagaatgtg gatttcccca 600
ggatattatt atttgtttac cggggganag gataactgtt tcncntattt taattgaaca 660
aactnaaaca aaanctaagg aaatcc 686

<210> 37
<211> 681
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(681)
<223> n = A,T,C or G

<400> 37
gagacanacn naacgtcang agaanaaaag angcatggaa cacaanccag gcncgatggc 60
caccttccca ccagcancca gcgcccccca gcngcccnca ngnccggang accangactc 120
cancctgnat caatctganc tctattcctg gcccatnccc acctcggagg tggangccgn 180
aaaggtcgca cnnncagaga agctgctgcc ancaccancc gcccccnncc tgncgggctn 240
nataggaaac tggtgaccnn gctgcanaat tcatacagga gcacgcgang ggcacnnnct 300
cacactgagt tnnngatgan gcctnaccan ggacctnccc cagcnnattg annacnggac 360
tgcggaggaa ggaagacccc gnacnggatc ctggccggcn tgccaccccc ccaccccctag 420
gattatnccc cttgactgag tctctgaggg gctaccccgaa cccgcctcca ttcccctacca 480
natnntgctc natcgggact gacangctgg ggatnggagg ggctatcccc cancatcccc 540

tnanaccaac agcnacngan natnggggct ccccngggtc ggngcaacnc tcctncaccc 600
cggcgcnggc cttcggtgnt gtcctccntc aacnaattcc naaanggcgg gccccccngt 660
ggactcctcn ttgttccctc c 681

<210> 38
<211> 687
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(687)
<223> n = A,T,C or G

<400> 38
canaaaaaaa aaaacatggc cgaaaccagn aagctgcgcg atggcgccac ggcccctctt 60
ctcccggcct gtgtccggaa ggtttccctc cgaggcgccc cggctcccgc aagcggagga 120
gagggcggga cntgccgggg ccggagctca naggccctgg ggccgcttcg ctctcccgcc 180
atcgcaaggg cggcgctaac ctnaggcctc cccgcaaagg tcccctnangc ggnggcggcg 240
gggggctgtg anaaccgcaa aaanaacgct gggcgcgcng cgaacccgtc cacccccgcg 300
aaggananac ttccacagan gcagcgtttc cacagcccan agccacnttt ctagggtgat 360
gcaccccagt aagttcctgn cggggaagct caccgctgtc aaaaaaanct ttcgctccac 420
cggcgcacna aggggangan ggcangangc tgccgccccc acaggtcatc tgatcacgtc 480
gcccgcccta nttctgcttt gtgaatctcc actttgttca acccccaccg ccgttctctc 540
ctccttgcgc cttcctctna ccttaanaac cagcttcctn tacccnatng tanttnctct 600
gcncnngtng aaattaattc ggtccnccgg aacctcttnc ctgtggcaac tgctnaaaga 660
aactgctgtt ctgnttactg cngtccc 687

<210> 39
<211> 695
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(695)
<223> n = A,T,C or G

<400> 39
actagtctgg cctacaatag tgtgattcat gtaggacttc tttcatcaat tcaaaacccc 60
tagaaaaacg tatacagatt atataagtag ggataagatt tctaacattt ctgggctctc 120
tgacccctgc gctagactgt ggaaagggag tattattata gtatacaaca ctgctgttgc 180
cttattagtt ataacatgat aggtgctgaa ttgtgattca caatttaaaa acactgtaat 240
ccaaactttt ttttttaact gtagatcatg catgtgaatg ttaatgttaa tttgttcaan 300
gttgttatgg gtagaaaaaa ccacatgcct taaaatttta aaaagcaggg cccaaacttа 360
ttagtttaaa attaggggta tgtttccagt ttgttattaa ntggttatag ctctgtttag 420
aanaaatcna ngaacangat ttngaaantt aagntgacat tatttnccag tgacttgtta 480
atttgaaatc anacacggca cctccgttt tggtnctatt ggnnttcgaa tccaancngg 540
ntccaaatct tnttggaaac ngtccnttta acttttttac nanatcttat ttttttattt 600
tggaatggcc ctatttaang ttaaaagggg ggggnnccac naccattcnt gaataaaact 650
naatatatat ccttggtccc ccaaaattta aggng 695

<210> 40
<211> 674
<212> DNA

<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(674)
<223> n = A,T,C or G

<400> 40
actagtagtc agttgggagt ggttgctata ccttgacttc atttatatga atttccactt 60
tattaaataa tagaaaagaa aatcccggtg cttgcagtag agttatagga cattctatgc 120
ttacagaaaa tatagccatg attgaaatca aatagtaaag gctgttctgg ctttttatct 180
tcttagctca tcttaaataa gtagtacact tgggatgcag tgcgtctgaa gtgctaatca 240
gttgtaacaa tagcacaaat cgaacttagg atgtgtttct tctcttctgt gtttcgattt 300
tgatcaattc tttaattttg ggaacctata atacagtttt cctattcttg gagataaaaa 360
ttaaatggat cactgatatt taagtcattc tgcttctcat ctnaatattc catattctgt 420
attagganaa antacctccc agcacagccc cctctcaaac cccacccaaa accaagcatt 480
tggaatgagt ctcctttatt tccgaantgt ggatggtata acccatatcn ctccaatttc 540
tgnttgggtt gggtattaat ttgaactgtg catgaaaagn ggnaatcttt nctttgggtc 600
aaantttncc ggttaatttg nctngncaaa tccaatttnc tttaagggtg tctttataaa 660
atttgctatt cngg 674

<210> 41
<211> 657
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(657)
<223> n = A,T,C or G

<400> 41
gaaacatgca agtaccacac actgtttgaa ttttgcacaa aaagtgactg tagggatcag 60
gtgatagccc cggaatgtac agtgtcttgg tgcaccaaga tgccttctaa aggctgacat 120
accttgggac cctaatgggg cagagagtat agccctagcc cagtggtgac atgaccactc 180
cctttgggag gctgaagtta aagggaatgg tatgtgtttt ctcatggaag cagcacatga 240
atnggtnaca ngatgttaaa ntaaggntct antttgggtg tcttgtcatt tgaaaaantg 300
acacactcct ancanctggt aaaggggtgc tggaagccat ggaagaactc taaaaacatt 360
agcatgggct gatctgatta cttcctggca tcccgctcac ttttatggga agtcttatta 420
naaggatggg anantttttcc atatccttgc tgttggaact ctggaacact ctctaaattt 480
ccctctatta aaaatcactg nccttactac acttcctcct tganggaata gaaatggacc 540
tttctctgac ttagttcttg gcatggganc cagcccaaat taaaatctga cttntccggt 600
ttctccngaa ctcacctact tgaattggta aaacctcctt tggaattagn aaaaacc 657

<210> 42
<211> 389
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(389)
<223> n = A,T,C or G

<400> 42

actagtgctg aggaatgtaa acaagtttgc tgggccttgc gagacttcac caggttgttt 60
cgatagctca cactcctgca ctgtgcctgt cacccaggaa tgtcttttttt aattagaaga 120
caggaagaaa acaaaaacca gactgtgtcc cacaatcaga aacctccgtt gtggcagang 180
ggccttcacc gccaccaggg tgtcccgcca gacagggaga gactccagcc ttctgaggcc 240
atcctgaaga attcctgttt gggggttgtg aaggaaaatc acccggattt aaaaagatgc 300
tgttgcctgc ccgcgtngtn gggaagggac tggtttcctg gtgaatttct taaaagaaaa 360
atattttaag ttaagaaaaa aaaaaaaaa 389

<210> 43
<211> 279
<212> DNA
<213> Homo sapien

<400> 43
actagtgaca agctcctggt cttgagatgt cttctcgtta aggagatggg ccttttggag 60
gtaaaggata aaatgaatga gttctgtcat gattcactat tctagaactt gcatgacctt 120
tactgtgtta gctcttttgaa tgttcttgaa attttagact ttctttgtaa acaaataata 180
tgtccttatc attgtataaa agctgttatg tgcaacagtg tggagatcct tgtctgattt 240
aataaaatac ttaaacactg aaaaaaaaaa aaaaaaaaa 279

<210> 44
<211> 449
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(449)
<223> n = A,T,C or G

<400> 44
actagtagca tcttttctac aacgttaaaa ttgcagaagt agcttatcat taaaaaacaa 60
caacaacaac aataacaata aatcctaagt gtaaatcagt tattctaccc cctaccaagg 120
atatcagcct gttttttccc ttttttctcc tgggaataat tgtgggcttc ttcccaaatt 180
tctacagcct ctttcctctt ctcatgcttg agcttccctg tttgcacgca tgcgttgtgc 240
aagantgggc tgtttngctt ggantncggt ccnagtggaa ncatgctttc ccttgttact 300
gttggaagaa actcaaacct tcnancccta ggtgttncca ttttgtcaag tcatcactgt 360
atttttgtac tggcattaac aaaaaaagaa atnaaatatt gttccattaa actttaataa 420
aactttaaaa gggaaaaaaa aaaaaaaaa 449

<210> 45
<211> 559
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(559)
<223> n = A,T,C or G

<400> 45
actagtgtgg gggaatcacg gacacttaaa gtcaatcttgc gaaataattc ttttattaca 60
cacttactga agtttttgag tcccagagag ccattctatg tcaaacattc caagtacttt 120
ttgagagccc agcattacat caacatgccc gtgcagttca aaccgaagtc cgcaggcaaa 180
tttgaagctt tgcttgtcat tcaaacagat gaaggcaaga gtattgctat tcgactaatt 240

ggtgaagctc ttggaaaaaa ttnactagaa tacttttttgt gttaagttaa ttacataagt 300
tgtattttgt taactttatc tttctacact acaattatgc ttttgtatat atattttgta 360
tgatggatat ctataattgt agattttgtt tttacaagct aatactgaag actcgactga 420
aatattatgt atctagccca tagtattgta cttaactttt acagggtgaa aaaaaaattc 480
tgtgtttgca ttgattatga tattctgaat aaatatggga atatatttta atgtgggtaa 540
aaaaaaaaaa aaaaaggaa 559

<210> 46
<211> 731
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(731)
<223> n = A,T,C or G

<400> 46
actagttcta gtaccatggc tgtcatagat gcaaccatta tattccattt agttttcttcc 60
tcaggttccc taacaattgt ttgaaactga atatatatgt ttatgtatgt gtgtgtgttc 120
actgtcatgt atatggtgta tatgggatgt gtgcagtttt cagttatata tatattcata 180
tatacatatg catatatatg tataatatac atatatacat gcatacactt gtataatata 240
catatatata cacatatatg cacacatatn atcactgagt tccaaagtga gtctttattt 300
ggggcaattg tatttttctcc ctctgtctgc tcactgggcc tttgcaagac atagcaattg 360
cttgatttcc tttggataag agtcttatct tcggcactct tgactctagc cttaactttа 420
gatttctatt ccagaatacc tctcatatct atcttaaaac ctaaganggg taaagangtc 480
ataagattgt agtatgaaag antttgctta gttaaattat atctcaggaa actcattcat 540
ctacaaatta aattgtaaaa tgatggtttg ttgtatctga aaaaatgttt agaacaagaa 600
atgtaactgg gtacctgtta tatcaaagaa cctcnatttа ttaagtctcc tcatagccan 660
atccttatat ngccctctct gacctgantt aatananact tgaataatga atagttaatt 720
taggnttggg c 731

<210> 47
<211> 640
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(640)
<223> n = A,T,C or G

<400> 47
tgcgngccgg tttggccctt ctttgtanga cactttcatc cgccctgaaa tcttcccgat 60
cgttaataac tcctcaggtc cctgcctgca cagggttttt tcttantttg ttgcctaaca 120
gtacaccaaa tgtgacatcc tttcaccaat atngattnct tcataccaca tcntcnatgg 180
anacgactnc aacaattttt tgatnacccn aaanactggg ggctnnaana agtacantct 240
ggagcagcat ggacctgtcn gcnactaang gaacaanagt nntgaacatt tacacaacct 300
ttggtatgtc ttactgaaag anagaaacat gcttctnncc ctagaccacg aggncaaccg 360
caganattgc caatgccaag tccgagcggt tagatcaggt aatacattcc atggatgcat 420
tacatacntt gtcccgaaa nanaagatgc cctaanggct tcttcanact ggtccngaaa 480
acanctacac ctggtgcttg ganaacanac tctttggaag atcatctggc acaagttccc 540
cccagtgggt tttncccttgg cacctanctt accanatcna ttcggaancc attctttgcc 600
ntggcnttnt nttgggacca ntctttctcac aactgnaccc 640

<210> 48
<211> 257
<212> DNA
<213> Homo sapien

<400> 48
actagtatat gaaaatgtaa atatcacttg tgtactcaaa caaaagttgg tcttaagctt 60
ccaccttgag cagccctgga aacctaacct gcctctttta gcataatcac attttctaaa 120
tgatttttctt tgttcctgaa aaagtgattt gtattagttt tacatttgtt ttttggaaga 180
ttatatttgt atatgtatca tcataaaata tttaaataaa aagtatcttt agagtgaaaa 240
aaaaaaaaaa aaaaaaa 257

<210> 49
<211> 652
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(652)
<223> n = A,T,C or G

<400> 49
actagttcag atgagtggct gctgaagggg cccccttgtc attttcatta taacccaatt 60
tccacttatt tgaactctta agtcataaat gtataatgac ttatgaatta gcacagttaa 120
gttgacacta gaaactgccc atttctgtat tacactatca aataggaaac attggaaaga 180
tggggaaaaa aatctttattt taaaatggct tagaaagttt tcagattact ttgaaaattc 240
taaacttctt tctgtttcca aaacttgaaa atatgtagat ggactcatgc attaagactg 300
ttttcaaagc tttcctcaca ttttttaaagt gtgatttttcc ttttaatata catatttatt 360
ttctttaaag cagctatatc ccaacccatg actttggaga tatacctatn aaaccaatat 420
aacagcangg ttattgaagc agctttctca aatgttgctt cagatgtgca agttgcaaat 480
tttattgtat ttgtanaata caatttttgt tttaaactgt atttcaatct atttctccaa 540
gatgcttttc atatagagtg aaatatccca ngataactgc ttctgtgtcg tcgcatttga 600
cgcataactg cacaaatgaa cagtgtatac ctcttggttg tgcattnacc cc 652

<210> 50
<211> 650
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(650)
<223> n = A,T,C or G

<400> 50
ttgcgctttg attttttag ggcttgtgcc ctgtttcact tatagggtct agaatgcttg 60
tgttgagtaa aaaggagatg cccaatattc aaagctgcta aatgttctct ttgccataaa 120
gactccgtgt aactgtgtga acacttggga ttttttctcct ctgtcccgag gtcgtcgtct 180
gcttttcttt ttgggttctt tctagaagat tgagaaatgc atatgacagg ctgagancac 240
ctcccaaac acacaagctc tcagccacan gcagcttctc cacagcccca gcttcgcaca 300
ggctcctgga nggctgcctg ggggaggcag acatgggagt gccaaggtgg ccagatggtt 360
ccaggactac aatgtcttta tttttaactg tttgccactg ctgccctcac ccctgcccgg 420
ctctggagta ccgtctgccc canacaagtg ggantgaaat gggggtgggg gggaacactg 480
attcccantt agggggtgcc taactgaaca gtagggatan aaggtgtgaa cctgngaant 540

gcttttataa attatnttcc ttgttanatt tatttttaa tttaatctct gttnaactgc 600
ccngggaaaa ggggaaaaaa aaaaaaaaat tctntttaaa cacatgaaca 650

<210> 51
<211> 545
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(545)
<223> n = A,T,C or G

<400> 51
tggcgtgcaa ccagggtagc tgaagtttgg gtctgggact ggagattggc cattaggcct 60
cctganattc cagctccctt ccaccaagcc cagtcttgct acgtggcaca gggcaaacct 120
gactcccttt gggcctcagt ttcccctccc cttcatgana tgaaaagaat actacttttt 180
cttgttggtc taacnttgct ggacncaaag tgtngtcatt attgttgtat tgggtgatgt 240
gtncaaaact gcagaagcta actgcctatg agaggaanta agagagatag tggatganag 300
ggacanaagg agtcattatt tggtatagat ccacccntcc caaccttttt ctcctcagtc 360
cctgcncctc atgtntctgg tntggtgagt cctttgtgcc accanccatc atgctttgca 420
ttgctgccat cctgggaagg gggtgnatcg tctcacaact tgttgtcatc gtttganatg 480
catgcttttt tnatnaaaca aanaaannaa tgtttgacag ngtttaaaat aaaaaanaaa 540
caaaa 545

<210> 52
<211> 678
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(678)
<223> n = A,T,C or G

<400> 52
actagtagaa gaactttgcc gcttttgtgc ctctcacagg cgcctaaagt cattgccatg 60
ggaggaagac gatttggggg gggagggggg gggggcangg tccgtggggc tttccctant 120
ntatctccat ntccantgnn cnntgtcgcc tcttccctcg tcncattnga anttantccc 180
tggncccccn ncccctctcn ncctncncct cccccctccg ncncctccnn ctttttntan 240
ncttccccat ctccntcccc cctnanngtc ccaacnccgn cagcaatnnc ncacttnctc 300
nctccncncc tccnnccgtt cttctnttct cnacntntnc ncnnntnccn tgccnntnaa 360
annctctccc cnctgcaanc gattctctcc ctccncnnan ctntccactc cntnctttcc 420
ncncgctcct nttcntcnnc ccaccctctn ccttcgnccc cantacnctc nccncccttn 480
cgnntcnttn nnntcctcnn accncccncc tcccttcncc cctcttctcc ccggtntntc 540
tctctcccnc nncncnncct cnncccntcc nngcgnccnt ttccgccccn cncncncntt 600
ccttcntcnc cantccatcn cntntncccat nctncctncc nctcacnccc gctncccccn 660
ntctcttca cacngtcc 678

<210> 53
<211> 502
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(502)
<223> n = A,T,C or G

<400> 53
tgaagatcct ggtgtcgcca tgggccgccg ccccgcccgt tgttaccggt attgtaagaa 60
caagccgtac ccaaagtctc gcttctgccg aggtgtcccc gatgccaaaa ttcgcatttt 120
tgacctgggg cggaaaaang caaaantgga tgagtctccg ctttgtggcc acatggtgtc 180
agatcaatat gagcagctgt cctctgaagc cctgnangct gcccgaattt gtgccaataa 240
gtacatggta aaaagtngtg gcnaagatgc ttccatatcc gggtgcggnt ccaccccttc 300
cacgtcatcc gcatcaacaa gatgttgtcc tgtgctgggg ctgacaggct cccaacaggc 360
atgcgaagtg cctttggaaa acccanggca ctgtggccag ggttcacatt gggccaattn 420
atcatgttca tccgcaccaa ctgcagaaca angaacntgt naattnaagc cctgcccagg 480
gncaanttca aatttcccgg cc 502

<210> 54
<211> 494
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(494)
<223> n = A,T,C or G

<400> 54
actagtccaa gaaaaatatg cttaatgtat attacaaagg ctttgtatat gttaaccctgt 60
tttaatgcca aaagtttgct ttgtccacaa tttccttaag acctcttcag aaagggattt 120
gtttgcctta atgaatactg ttgggaaaaa acacagtata atgagtgaaa agggcagaag 180
caagaaattt ctacatctta gcgactccaa gaagaatgag tatccacatt tagatggcac 240
attatgagga ctttaatctt tccttaaaca caataatgtt ttctttttttc ttttattcac 300
atgatttcta agtatatttt tcatgcagga cagttttttca accttgatgt acagtgactg 360
tgttaaattt ttcttttcagt ggcaacctct ataatcttta aaatatggtg agcatcttgt 420
ctgttttgaa ngggatatga cnatnaatct atcagatggg aaatcctgtt tccaagttag 480
aaaaaaaaaa aaaa 494

<210> 55
<211> 606
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(606)
<223> n = A,T,C or G

<400> 55
actagtaaaa agcagcattg ccaaataatc cctaattttc cactaaaaat ataatgaaat 60
gatgttaagc tttttgaaaa gtttaggtta aacctactgt tgttagatta atgtatttgt 120
tgcttcccct tatctggaat gtggcattag ctttttttatt ttaaccctct ttaattctta 180
ttcaattcca tgacttaagg ttggagagct aaacactggg atttttggat aacagactga 240
cagttttgca taattataat cggcattgta catagaaagg atatggctac cttttgttaa 300
atctgcactt tctaaatatc aaaaaaggga aatgaagtat aaatcaattt ttgtataatc 360
tgtttgaaac atgantttta tttgcttaat attanggctt tgccctttta tgttagtctc 420
ttgggatcct gtgtaaaact gttctcatta aacaccaaac agttaagtcc attctttggt 480

actagctaca aattccgttt catattctac ntaacaattt aaattaactg aaatatttct 540
anatggtcta cttctgtcnt ataaaaacna aacttgantt nccaaaaaaa aaaaaaaaaa 600
aaaaaa 606

<210> 56
<211> 183
<212> DNA
<213> Homo sapien

<400> 56
actagtatat ttaaacttac aggcttattt gtaatgtaaa ccaccatttt aatgtactgt 60
aattaacatg gttataatac gtacaatcct tccctcatcc catcacacaa ctttttttgt 120
gtgtgataaa ctgattttgg tttgcaataa aaccttgaaa aataaaaaaa aaaaaaaaaa 180
aaa 183

<210> 57
<211> 622
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(622)
<223> n = A,T,C or G

<400> 57
actagtcact actgtctttt ccttgtagct aatcaatcaa tatttttccc ttgcctgtgg 60
gcagtggaga gtgctgctgg gtgtacgctg cacctgccca ctgagttggg gaaagaggat 120
aatcagtgag cactgttctg ctcagagctc ctgatctacc ccacccccta ggatccagga 180
ctgggtcaaa gctgcatgaa accaggcccc ggcagcaacc tgggaatggc tggaggtggg 240
agagaacctg acttctcttt ccctctcccc cctccaacat tactggaact ctatcctgtt 300
agggatcttc tgagcttgtt tccctgctgg gtgggacaga agacaaagga gaagggangg 360
tctacaanaa gcagcccttc tttgtcctct ggggttaatg agcttgacct ananttcatg 420
gaganaccan aagcctctga tttttaattt ccntnaaatg tttgaagtnt atatntacat 480
atatatattt ctttnaatnt ttgagtcttt gatatgtctt aaaatccant ccctctgccn 540
gaaacctgaa ttaaaaccat gaanaaaaat gtttncctta aagatgttan taattaattg 600
aaacttgaaa aaaaaaaaaa aa 622

<210> 58
<211> 433
<212> DNA
<213> Homo sapien

<400> 58
gaacaaattc tgattggtta tgtaccgtca aaagacttga agaaatttca tgattttgca 60
gtgtggaagc gttgaaaatt gaaagttact gcttttccac ttgctcatat agtaaaggga 120
tcctttcagc tgccagtgtt gaataatgta tcatccagag tgatgttatc tgtgacagtc 180
accagcttta agctgaacca ttttatgaat accaaataaa tagacctctt gtactgaaaa 240
catatttgtg actttaatcg tgctgcttgg atagaaatat ttttactggc tcttctgaat 300
tgacagtaaa cctgtccatt atgaatggcc tactgttcta ttatttgttt tgacttgaat 360
ttatccacca aagacttcat ttgtgtatca tcaataaagt tgtatgtttc aactgaaaaa 420
aaaaaaaaaa aaa 433

<210> 59
<211> 649

<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(649)
<223> n = A,T,C or G

<400> 59
actagttatt atctgacttt cnggttataa tcattctaat gagtgtgaag tagcctctgg 60
tgtcatttgg atttgcattt ctctgatgag tgatgctatc aagcaccttt gctggtgctg 120
ttggccatat gtgtatgttc cctggagaag tgtctgtgct gagccttggc ccactttta 180
attaggcgtn tgtcttttta ttactgagtt gtaaganttc tttatatatt ctggattcta 240
gacccttatc agatacatgg tttgcaaata ttttctccca ttctgtgggt tgtgttttca 300
ctttatcgat aatgtcctta gacatataat aaatttgtat tttaaaagtg acttgatttg 360
ggctgtgcaa ggtgggctca cgcttgtaat cccagcactt tgggagactg aggtgggtgg 420
atcatatgan gangctagga gttcgaggtc agcctggcca gcatagcgaa aacttgtctc 480
tacnaaaaat acaaaaatta gtcaggcatg gtggtgcacg tctgtaatac cagctttca 540
ggangctgan gcacaaggat cacttgaacc ccagaangaa gangttgcag tganctgaag 600
atcatgccag ggcaacaaaa atgagaactt gtttaaaaaa aaaaaaaaa 649

<210> 60
<211> 423
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(423)
<223> n = A,T,C or G

<400> 60
actagttcag gctttccagt tcactgacaa acatggggaa gtgtgcccag ctggctggaa 60
acctggcagt gataccatca agcctgatgt ccaaaagagc aaagaatatt tctccaagca 120
gaagtgagcg ctgggctgtt ttagtgccag gctgcggtgg gcagccatga gaacaaaacc 180
tcttctgtat tttttttttc cattagtana acacaagact cngattcagc cgaattgtgg 240
tgtcttacaa ggcagggctt tcctacaggg ggtgganaaa acagcctttc ttcctttggt 300
aggaatggcc tgagttggcg ttgtgggcag gctactggtt tgtatgatgt attagtagag 360
caacccatta atcttttgta gtttgtatna aacttganct gagaccttaa acaaaaaaaa 420
aaa 423

<210> 61
<211> 423
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(423)
<223> n = A,T,C or G

<400> 61
cgggactgga atgtaaagtg aagttcggag ctctgagcac gggctctttc cgccgggtcc 60
tccctcccca gatttcagag ggagaggccc acccccgccca gccccgcccc agccccctgct 120
caggtctgag tatggctggg agtcgggggc cacaggcctc tagctgtgct gctcaagaag 180

actggatcag ggtanctaca agtggccggg ccttgccttt gggattctac cctgttccta 240
atttggtgtt ggggtgcggg gtcccctggcc ccctttttcca cactncctcc ctccngacag 300
caacctcccc tggggcaatt gggcctggnt ctccncccgn tgttgcnacc ctttgttggt 360
ttaaggnctt taaaaatgtt annttttccc ntgccngggt taaaaaagga aaaaactnaa 420
aaa 423

<210> 62
<211> 683
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(683)
<223> n = A,T,C or G

<400> 62
gctggagagg ggtacggact ttcttggagt tgtcccaggt tggaatgaga ctgaacttaa 60
gaagagaccc taagagactg gggaatggtt cctgccttca ggaaagtgaa agacgcttag 120
gctgtcaaca cttaaaggaa gtccccttga agcccagagt ggacagacta gacccattga 180
tggggccact ggccatggtc cgtggacaag acattccngt gggccatggc acaccggggg 240
ggatcaaaat gtgtacttgt ggggtcttgc cccttgccaa aaccaaacca ntcccactnc 300
tgtcnttgga cttttcttccc attcccctcct cccaaaatgc acttcccccc ctcccctctgc 360
ccctcctgtg tttttggaat tctgtttccc tcaaaattgt taatttttta nttttngacc 420
atgaacttat gtttgggggtc nangttcccc ttnccaatgc atactaatat attaatggtt 480
atttatttct gaaatatttt ttaatgaact tggaaaaaat tnntggaatt tcctttnctnc 540
cnttttnttt ggggggggtg gggggntggg ttaaaatttt tttggaancc cnatnggaaa 600
ttnttacttg gggcccccct naaaaaantn anttccaatt cttnnatngc ccctnttccn 660
ctaaaaaaaa ananannaaa aan 683

<210> 63
<211> 731
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(731)
<223> n = A,T,C or G

<400> 63
actagtcata aagggtgtgc gcgtcttcga cgtggcggtc ttggcgccac tgctgcgaga 60
cccggccctg gacctcaagg tcatccactt ggtgcgtgat ccccgcgcgg tggcgagttc 120
acggatccgc tcgcgccacg gcctcatccg tgagagccta caggtggtgc gcagccgaga 180
ccgcgagctc accgcatgcc cttcttggag gccgcgggcc acaagcttgg cgcccanaaa 240
gaaggcgtng ggggcccgca aantaccacg ctctgggcgc tatggaangt cctcttgcaa 300
taatattggt tnaaaanctg canaanagcc cctgcanccc cctgaactgg gntgcagggc 360
cncttacctn gtttggntgc ggttacaaag aacctgtttn ggaaaaccct nccnaaaacc 420
ttccgggaaa attntncaaa ttttttnttgg ggaattnttg ggtaaacccc ccnaaaatgg 480
gaaacntttt tgccctnnaa antaaaccat tnggttccgg gggccccccc ncaaaaccct 540
ttttttnttt ttttntgccc cantnncccc ccggggcccc tttttttngg ggaaaanccc 600
ccccctncc nanantttta aaagggnggg anaatttttn nttnccccnc gggnccccnn 660
ggngntaaaa nggtttcncc cccccgaggg gnggggnnnc ctcnnaaacc cntntcnnna 720
ccncnttttn n 731

<210> 64
<211> 313
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(313)
<223> n = A,T,C or G

<400> 64
actagttgtg caaaccacga ctgaagaaag acgaaaagtg ggaaataact tgcaacgtct 60
gttagagatg gttgctacac atgttgggtc tgtagagaaa catcttgagg agcagattgc 120
taaagttgat agagaatatg aagaatgcat gtcagaagat ctctcggaaa atattaaaga 180
gattagagat aagtatgaga agaaagctac tctaattaag tcttctgaag aatgaagatn 240
aaatgttgat catgtatata tatccatagt gaataaaatt gtctcagtaa agttgtaaaa 300
aaaaaaaaaa aaa 313

<210> 65
<211> 420
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(420)
<223> n = A,T,C or G

<400> 65
actagttccc tggcaggcaa gggcttccaa ctgaggcagt gcatgtgtgg cagagagagg 60
caggaagctg gcagtggcag cttctgtgtc tagggagggg tgtggctccc tccttccctg 120
tctgggaggt tggagggaag aatctaggcc ttagcttgcc ctcctgccac ccttcccctt 180
gtagatactg ccttaacact ccctcctctc tcagctgtgg ctgccatcca agccaggttt 240
ctccgtgctc actaatttat ttccaggaaa ggtgtgtgga agacatgagc cgtgtataat 300
atttgtttta acattttcat tgcaagtatt gaccatcatc cttggttgtg tatcgttgta 360
acacaaatta atgatattaa aaagcatcca aacaaagccn annnnnaana nnannngaaa 420

<210> 66
<211> 676
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(676)
<223> n = A,T,C or G

<400> 66
actagtttcc tatgatcatt aaactcattc tcagggttaa gaaaggaatg taaatttctg 60
cctcaatttg tacttcatca ataagttttt gaagagtgca gatttttagt caggtcttaa 120
aaataaactc acaaatctgg atgcatttct aaattctgca aatgtttcct ggggtgactt 180
aacaaggaat aatcccacaa tatacctagc tacctaatac atggagctgg ggcttaaccc 240
actgttttta aggatttgcg cttacttgtg gctgaggaaa aataagtagt tccgagggaa 300
gtagttttta aatgtgagct tatagatngg aaacagaata tcaacttaat tatggaaatt 360
gttagaaacc tgtttctctg ttatctgaat cttgattgca attactattg tactggatag 420

actccagccc attgcaaagt ctcagatatc ttanctgtgt agttgaattc cttggaaatt 480
ctttttaaga aaaaattgga gtttnaaaga aataaacccc tttgttaaat gaagcttggc 540
tttttggtga aaaanaatca tcccgcaggg cttattgttt aaaaanggaa ttttaagcct 600
ccctggaaaa anttgttaat taaatgggga aaatgntggg naaaaattat ccgttagggt 660
ttaaagggaa aactta 676

<210> 67
<211> 620
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(620)
<223> n = A,T,C or G

<400> 67
caccattaaa gctgcttacc aagaacttcc ccagcatttt gacttccttg tttgatagct 60
gaattgtgag caggtgatag aagagccttt ctagttgaac atacagataa tttgctgaat 120
acattccatt taatgaaggg gttacatctg ttacgaagct actaagaagg agcaagagca 180
taggggaaaa aaattctgatc agaacgcatc aaacttcacat gtgccccctc tactacaaac 240
agattgtagt gctgtggtgg tttattccgt tgtgcagaac ttgcaagctg agtcactaaa 300
cccaaagaga ggaaattata ggttagttaa acattgtaat cccaggaact aagtttaatt 360
cacttttgaa gtgttttgtt ttttatttt ggtttgtctg atttactttg ggggaaaang 420
ctaaaaaaaa agggatatca atctctaatt cagtgcccac taaaagttgt ccctaaaaag 480
tctttactgg aanttatggg actttttaag ctccaggtnt tttggtcctc caaattaacc 540
ttgcatgggc ccctttaaaat tgttgaangg cattcctgcc tctaagtttg gggaaaattc 600
ccccnttttn aaaatttgga 620

<210> 68
<211> 551
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(551)
<223> n = A,T,C or G

<400> 68
actagtagct ggtacataat cactgaggag ctatttctta acatgctttt atagaccatg 60
ctaatgctag accagtattt aagggctaat ctcacacctc cttagctgta agagtctggc 120
ttagaacaga cctctctgtg caataacttg tggccactgg aaatccctgg gccggcattt 180
gtattggggt tgcaatgact cccaagggcc aaaagagtta aaggcacgac tgggatttct 240
tctgagactg tggtgaaact ccttccaagg ctgaggggct cagtangtgc tctgggaggg 300
actcggcacc actttgatat tcaacaagcc acttgaagcc caattataaa attgttattt 360
tacagctgat ggaactcaat ttgaaccttc aaaactttgt tagtttatcc tattatattg 420
ttaaacctaa ttacatttgt ctagcattgg atttggttcc tgtngcatat gtttttttcn 480
cctatgtgct ccccttcccc nnatcttaat ttaaaccnca attttgcnat tcncccnnnnn 540
nannnannna a 551

<210> 69
<211> 396
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(396)
<223> n = A,T,C or G

<400> 69
cagaaatgga aagcagagtt ttcatttctg tttataaacg tctccaaaca aaaatggaaa 60
gcagagtttt cattaaatcc ttttaccttt ttttttttct ggtaatcccc tcaaataaca 120
gtatgtggga tattgaatgt taaagggata ttttttttcta ttatttttat aattgtacaa 180
aattaagcaa atgttaaaag ttttatatgc tttattaatg ttttcaaaag gtatnataca 240
tgtgatacat tttttaagct tcagttgctt gtcttctggt actttctgtt atgggctttt 300
ggggagccan aaaccaatct acnatctctt tttgtttgcc aggacatgca ataaaatttа 360
aaaaataaat aaaaactatt nagaaattga aaaaaa 396

<210> 70
<211> 536
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(536)
<223> n = A,T,C or G

<400> 70
actagtgcaa aagcaaatat aaacatcgaa aaggcgttcc tcacgttagc tgaagatatc 60
ctttgaaaga cccctgtaaa agagcccaac agtgaaaatg tagatatcag cagtggagga 120
ggcgtgacag gctggaagag caaatgctgc tgagcattct cctgttccat cagttgccat 180
ccactacccc gttttctctt cttgctgcaa aataaaccac tctgtccatt tttaactcta 240
aacagatatt tttgtttctc atcttaacta tccaagccac ctattttatt tgttctttca 300
tctgtgactg cttgctgact ttatcataat tttcttcaaa caaaaaaatg tatagaaaaa 360
tcatgtctgt gacttcattt ttaaatgnta cttgctcagc tcaactgcat ttcagttgtt 420
ttatagtcca gttcttatca acattnaaac ctatngcaat catttcaaat ctattctgca 480
aattgtataa gaataaaagt tagaatttaa caattaaaaa aaaaaaaaaa aaaaaa 536

<210> 71
<211> 865
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(865)
<223> n = A,T,C or G

<400> 71
gacaaagcgt taggagaaga anagaggcag ggaanactnc ccaggcacga tggccncctt 60
cccaccagca accagcgccc cccaccagcc cccaggcccg gacgacgaag actccatcct 120
ggattaatct nacctctntc gcctgncccа ttcctacctc ggaggtggag gccggaaagg 180
tcncaccaag aganaanctg ctgccaacac caaccgcccc agcccctggcg ggcacganag 240
gaaactggtg accaatctgc agaattctna gaggaanaag cnaggggccc cgcgctnaga 300
cagagctgga tatgangcca gaccatggac nctacncccn ncaatncana cgggactgcg 360
gaagatggan gacccncgac nngatcaggc cngctnncca nccccccacc cctatgaatt 420
attcccgctg aangaatctc tgannggctt ccannaaagc gcctcccccnc cnaacgnaan 480

tncaacatng ggattanang ctgggaactg naaggggcaa ancctnnaat atccccagaa 540
acaanctctc ccnaanaaac tggggcncct catnggtggn accaactatt aactaaaccg 600
cacgccaagn aantataaaa gggggggccc tccncggnng acccccctttt gtcccttaat 660
ganggttatc cncctgcgt accatggtnc ccnnttctgt ntgnatgttt ccnctcccct 720
ccnccctatnt cnagccgaac tcnnatttnc ccgggggtgc natcnantng tncnccttcn 780
ttngttgncc cngccctttc cgncggaacn cgtttccccg ttantaacgg cacccggggn 840
aagggtgntt ggccccctcc ctccc 865

<210> 72
<211> 560
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(560)
<223> n = A,T,C or G

<400> 72
cctggacttg tcttggttcc agaacctgac gacccggcga cggcgacgtc tcttttgact 60
aaaagacagt gtccagtgct ccngcctagg agtctacggg gaccgcctcc cgcgccgcca 120
ccatgcccaa cttctctggc aactggaaaa tcatccgatc ggaaaacttc gangaattgc 180
tcnaantgct gggggtgaat gtgatgctna ngaanattgc tgtggctgca gcgtccaagc 240
cagcagtgga gatcnaacag gagggagaca ctttctacat caaaacctcc accaccgtgc 300
gcaccacaaa gattaacttc nnngttgggg agganttttga ggancaaact gtggatngga 360
ngcctgtnaa aacctggtga aatgggagaa tganaataaa atggtctgtg ancanaaact 420
cctgaaagga gaaggccccc anaactcctg gaccngaaaa actgacccnc cnatnggggа 480
actgatnctt gaaccctgaa cgggcgggat gancctttct tnttgccncc naangggttc 540
tttccntttc cccaaaaaaa 560

<210> 73
<211> 379
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(379)
<223> n = A,T,C or G

<400> 73
ctggggancc ggcggtnngc nccatntcnn gncgcgaagg tggcaataaa aanccnctga 60
aaccgcncaa naaacatgcc naagatatgg acgaggaaga tngngctttc nngnacaanc 120
gnannngagga acanaacaaa ctcnangagc tctcaagcta atgccgcggg gaaggggccc 180
ttggccacnn gtggaattaa gaaatctggc aaanngtann tgttccttgt gcctnangag 240
ataagngacc ctttatttca tctgtattta aaccctctcn ttcccctgnca taacttcttt 300
tnccacgtan agntggaant anttgttgtc ttggactgtt gtncatttta gannaaactt 360
ttgttcaaaa aaaaaataa 379

<210> 74
<211> 437
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(437)
<223> n = A,T,C or G

<400> 74
actagttcag actgccacgc caaccccaga aaatacccca catgccagaa aagtgaagtc 60
ctaggtgttt ccatctatgt ttcaatctgt ccatctacca ggcctcgcga taaaaacaaa 120
acaaaaaaac gctgccaggt tttanaagca gttctggtct caaaaccatc aggatcctgc 180
caccagggtt cttttgaaat agtaccacat gtaaaaggga atttggcttt cacttcatct 240
aatcactgaa ttgtcaggct ttgattgata attgtagaaa taagtagcct tctgttgtgg 300
gaataagtta taatcagtat tcatctcttt gttttttgtc actcttttct ctctnattgt 360
gtcatttgta ctgtttgaaa aatatttctt ctataaaatt aaactaacct gccttaaaaa 420
aaaaaaaaaa aaaaaaa 437

<210> 75
<211> 579
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(579)
<223> n = A,T,C or G

<400> 75
ctccgtcgcc gccaagatga tgtgcggggc gcccttcgcc acgcagccgg ccaccgccga 60
gacccagcac atcgccgacc aggtgaggtc ccagcttgaa gagaaagaaa acaagaagtt 120
ccctgtgttt aaggccgtgt cattcaagag ccaggtggtc gcggggacaa actactttcat 180
caaggtgcac gtcggcgacg aggacttcgt acacctgcga gtgttccaat ctctcccttca 240
tgaaaacaag ccattgacct tatctaacta ccagaccaac aaagccaagc atgatgagct 300
gacctatttc tgatcctgac tttggacaag gcccttcagc cagaagactg acaaagtcat 360
cctccgtcta ccagagcgtg cacttgtgat cctaaaataa gcttcatctc cgggctgtgc 420
ccttggggtg gaaggggcan gatctgcact gcttttgcat ttctcttcct aaatttcatt 480
gtgttgattc tttccttcca ataggtgatc ttnattactt tcagaatatt ttccaaatna 540
gatatatttt naaaatcctt aaaaaaaaaa aaaaaaaaa 579

<210> 76
<211> 666
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(666)
<223> n = A,T,C or G

<400> 76
gtttatccta tcttttcaac cagattgtca gctccttgag ggcaagagcc acagtatatt 60
tccctgtttc ttccacagtg cctaataata ctgtggaact aggttttaat aatttttttaa 120
ttgatgttgt tatgggcagg atggcaacca gaccattgtc tcagagcagg tgctggctct 180
ttcctggcta ctccatgttg gctagcctct ggtaaccttt tacttattat cttcaggaca 240
cttcactacag ggaccaggga tgatgcaaca tccttgtctt tttatgacag gatgtttgct 300
cagcttcttcc aacaataaaa agcacgtggt aaaacacttg cggatatttct ggactgtttt 360
taaaaaaatat acagtttacc gaaaatcata ttatcttaca atgaaaagga ntttatagat 420
cagccagtga acaacctttt tccaccatac aaaaattcct tttcccgaan gaaaanggct 480

ttctcaataa ncctcacttt cttaanatct tacaagatag ccccganatc ttatcgaaac 540
tcattttagg caaatatgan ttttattgtn cgttacttgt ttcaaaattt ggtattgtga 600
atatcaatta ccacccccat ctccatgaa anaaanggga aanggtgaan ttcntaancg 660
cttaaa 666

<210> 77
<211> 396
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(396)
<223> n = A,T,C or G

<400> 77
ctgcagcccg ggggatccac taatctacca nggttatttg gcagctaatt ctanatttgg 60
atcattgccc aaagttgcac ttgctggtct cttgggattt ggccttggaa aggtatcata 120
catanganta tgccanaata aattccattt ttttgaaaat canctccntg gggctggttt 180
tggtccacag cataacangc actgcctcct tacctgtgag gaatgcaaaa taaagcatgg 240
attaagtgag aagggagact ctcagccttc agcttcctaa attctgtgtc tgtgactttc 300
gaagtttttt aaacctctga atttgtacac atttaaaatt tcaagtgtac tttaaaataa 360
aatacttcta atgggaacaa aaaaaaaaaa aaaaaa 396

<210> 78
<211> 793
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(793)
<223> n = A,T,C or G

<400> 78
gcatcctagc cgccgactca cacaaggcag gtgggtgagg aaatccagag ttgccatgga 60
gaaaattcca gtgtcagcat tcttgctcct tgtggccctc tcctacactc tggccagaga 120
taccacagtc aaacctggag ccaaaaagga cacaaaggac tctcgaccca aactgcccca 180
gacccctccc agaggttggg gtgaccaact catctggact cagacatatg aagaagcctt 240
atataaatcc aagacaagca acaaaccctt gatgattatt catcacttgg atgagtgccc 300
acacagtcna gctttaaaga aagtgtttgc tgaaaataaa gaaatccaga aattggcaga 360
gcagtttgtc ctcctcaatc tggtttatga aacaactgac aaacaccttt ctcctgatgg 420
ccagtatgtc ccaggattat gtttgttgac ccatctctga cagttgaagc cgatatcctg 480
ggaagatatt cnaaccgtct ctatgcttac aaactgcaga tacgctctgt tgcttgacac 540
atgaaaaagc tctcaagttg ctnaaaatga attgtaagaa aaaaaatctc cagcctttg 600
tctgtcggct tgaaaattga aaccagaaaa atgtgaaaaa tggctattgt ggaacanatn 660
gacacctgat taggttttgg ttatgttcac cactattttt aanaaaanan nttttaaaat 720
ttggttcaat tntctttttn aaacaatntg tttctacntt gnganctgat ttctaaaaaa 780
aataatnttt ggc 793

<210> 79
<211> 456
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(456)
<223> n = A,T,C or G

<400> 79
actagtatgg ggtgggaggc cccacccttc tcccctaggc gctgttcttg ctccaaaggg 60
ctccgtggag agggactggc agagctgang ccacctgggg ctggggatcc cactcttctt 120
gcagctgttg agcgcaccta accactggtc atgcccccac ccctgctctc cgcacccgct 180
tcctcccgac cccangacca ggctacttct cccctcctct tgcctccctc ctgcccctgc 240
tgcctctgat cgtangaatt gangantgtc ccgccttgtg gctganaatg gacagtggca 300
ggggctggaa atgggtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gcnccccccc 360
tgcaagaccg agattgaggg aaancatgtc tgctgggtgt gaccatgttt cctctccata 420
aantncccct gtgacnctca naaaaaaaaa aaaaaa 456

<210> 80
<211> 284
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(284)
<223> n = A,T,C or G

<400> 80
ctttgtacct ctagaaaaga taggtattgt gtcatgaaac ttgagtttaa attttatata 60
taaaactaaa agtaatgctc actttagcaa cacatactaa aattggaacc atactgagaa 120
gaatagcatg acctccgtgc aaacaggaca agcaaatttg tgatgtgttg attaaaaaga 180
aataaataaa tgtgtatatg tgtaacttgt atgtttatgt ggaatacaga ttgggaaata 240
aaatgtattt cttactgtga aaaaaaaaaa aaaaaaaaaa aana 284

<210> 81
<211> 671
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(671)
<223> n = A,T,C or G

<400> 81
gccaccaaca ttccaagcta ccctgggtac ctttgtgcag tagaagctag tgagcatgtg 60
agcaagcggt gtgcacacgg agactcatcg ttataattta ctatctgcca agagtagaaa 120
gaaaggctgg ggatatttgg gttggcttgg ttttgatttt ttgcttgttt gtttgttttg 180
tactaaaaca gtattatctt ttgaatatcg tagggacata agtatataca tgttatccaa 240
tcaagatggc tagaatggtg cctttctgag tgtctaaaac ttgacacccc tggtaaatct 300
ttcaacacac ttccactgcc tgcgtaatga agttttgatt catttttaac cactggaatt 360
ttttaatgcc gtcatttttca gttagatnat tttgcacttt gagattaaaa tgccatgtct 420
atttgattag tctttatttt ttatttttac aggcttatca gtctcactgt tggctgtcat 480
tgtgacaaag tcaaataaac ccccnaggac aacacacagt atgggatcac atattgtttg 540
acattaagct ttggccaaaa aatgttgcat gtgttttacc tcgactttgct aaatcaatan 600
canaaaggct ggccnataat gttggtggtg aaataattaa tnantaacca aaaaaaaaan 660
aaaaaaaaaa a 671

<210> 82
<211> 217
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(217)
<223> n = A,T,C or G

<400> 82
ctgcagatgt ttcttgaatg ctttgtcaaa ttaanaaagt taaagtgcaa taatgtttga 60
agacaataag tggtggtgta tcttgtttct aataagataa actttttgt ctttgcttta 120
tcttattagg gagttgtatg tcagtgtata aaacatactg tgtggtataa caggcttaat 180
aaattcttta aaaggaaaaa aaaaaaaaaa aaaaaaa 217

<210> 83
<211> 460
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(460)
<223> n = A,T,C or G

<400> 83
cgcgagtggg agcaccagga tctcgggctc ggaacgagac tgcacggatt gttttaagaa 60
aatggcagac aaaccagaca tgggggaaat cgccagcttc gatnaggcca agctgaanaa 120
aacggagacg caggagaaga acaccctgcc gaccaaagag accattgagc angagaagcg 180
gagtgaaatt tcctaagatc ctggaggatt tcctaccccc gtcctcttcg agaccccagt 240
cgtgatgtgg aggaagagcc acctgcaaga tggacacgag ccacaagctg cactgtgaac 300
ctgggcactc cgcgccgatg ccaccggcct gtgggtctct gaagggaccc ccccaatcg 360
gactgccaaa ttctccggtt tgccccggga tattatacaa nattatttgt atgaataatg 420
annataaaac acacctcgtg gcancaaana aaaaaaaaaa 460

<210> 84
<211> 323
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(323)
<223> n = A,T,C or G

<400> 84
tggtggatct tggctctgtg gagctgctgg gacgggatct aaaagactat tctggaagct 60
gtggtccaan gcattttgct ggcttaacgg gtcccggaac aaaggacacc agcttctaa 120
aattgaagtt taccgganat aacaatcttt tgggcagaga tgcctatttt aacaaacncc 180
gtcccctgcgc aacaacnaac aatctctggg aaataccggc catgaacntg ctgttcaat 240
cnancatctc tctagctgac cgatcatatc gtcccagatt actacanatc ataataattg 300
atttcctgta naaaaaaaaa aaa 323

<210> 85
<211> 771
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(771)
<223> n = A,T,C or G

<400> 85
aaactgggta ctcaacactg agcagatctg ttctttgagc taaaaaccat gtgctgtacc 60
aanagtttgc tcctggctgc tttgatgtca gtgctgctac tccacctctg cggcgaatca 120
gaagcaagca actttgactg ctgtcttgga tacacagacc gtatttttca tcctaaattt 180
attgtgggct tcacacggca gctggccaat gaaggctgtg acatcaatgc tatcatcttt 240
cacacaaaga aaaagttgtc tgtgtgcgca aatccaaaac agacttgggt gaaatatatt 300
gtgcgtctcc tcagtaaaaa agtcaagaac atgtaaaaac tgtggctttt ctggaatgga 360
attggacata gcccaagaac agaaagaact tgctggggtt ggaggttttca cttgcacatc 420
atgganggtt tagtgcttat cttatttgtg cctcctggac ttgtccaatt natgaagtta 480
atcatattgc atcatanttt gctttgttta acatcacatt naaattaaac tgtattttat 540
gttatttata gctntaggtt ttctgtgttt aacttttttat acnaantttc ctaaactatt 600
ttggtntant gcaanttaaa aattatattt ggggggggaa taaatattgg antttctgca 660
gccacaagct ttttttaaaa aaccantaca nccnngttaa atggtnggtc ccnaatggtt 720
tttgcttttn antagaaaat ttnttagaac natttgaaaa aaaaaaaaaa a 771

<210> 86
<211> 628
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(628)
<223> n = A,T,C or G

<400> 86
actagtttgc tttacatttt tgaaaagtat tatttttgtc caagtgctta tcaactaaac 60
cttgtgttag gtaagaatgg aatttattaa gtgaatcagt gtgacccttc ttgtcataag 120
attatcttaa agctgaagcc aaaatatgct tcaaaagaaa angactttat tgttcattgt 180
agttcataca ttcaaagcat ctgaactgta gttttctatag caagccaatt acatccataa 240
gtggagaang aaatagatta atgtcnaagt atgattggtg gagggagcaa ggttgaagat 300
aatctggggt tgaaattttc tagttttcat tctgtacatt tttagttnga catcagattt 360
gaaatattaa tgtttacctt tcaatgtgtg gtatcagctg gactcantaa cacccctttc 420
ttcccttnggg gatggggaat ggattattgg aaaatggaaa gaaaaaagta cttaaagcct 480
tcctttcnca gtttctggct cctaccctac tgatttancc agaataagaa aacattttat 540
catcntctgc tttattccca ttaatnaant tttgatgaat aaatctgctt ttatgcnnac 600
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<210> 87
<211> 518
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature

<223> n = A,T,C or G

<400> 87

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agtagtacag ttttaaaatt ttatgcttaa aacaagtttt gtgtaaaaaa tgcagataca     180
ttttacatgg caaatcaatt tttaagtcat cctaaaaatt gatttttttt tgaaatttaa     240
aaacacattt aatttcaatt tctctcttat ataaccttta ttactatagc atggtttcca     300
ctacagttta acaatgcagc aaaattccca tttcacggta aattgggttt taagcggcaa     360
ggttaaaatg ctttgaggat cctnaatacc ctttgaactt caaatgaagg ttatggttgt     420
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taaaancgag ccccccgttg aaaaagcaaa agggaccc                             518
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<210> 88
<211> 1844
<212> DNA
<213> Homo sapien

<400> 88

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ggtatttgct aaagcatttt gagctgcttg gaaaaaggga agtagttgca gtagagtttc     180
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<211> 523
<212> DNA

<222> (1)...(518)